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Intracerebral Transplantation of Malignant New Growths.

by

C. DA FANO, M.D.

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Fig. 1.

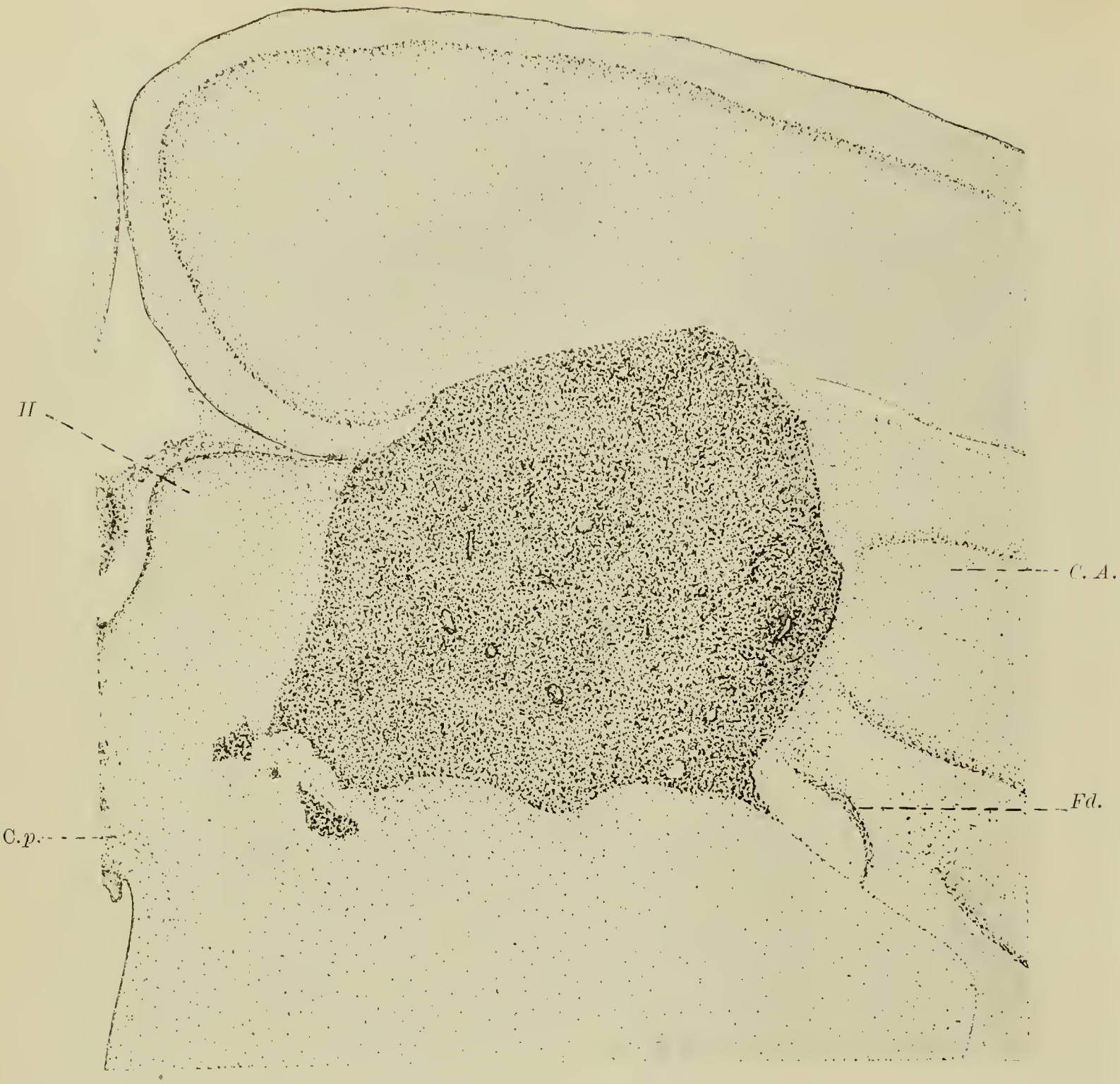

Fig. 3.

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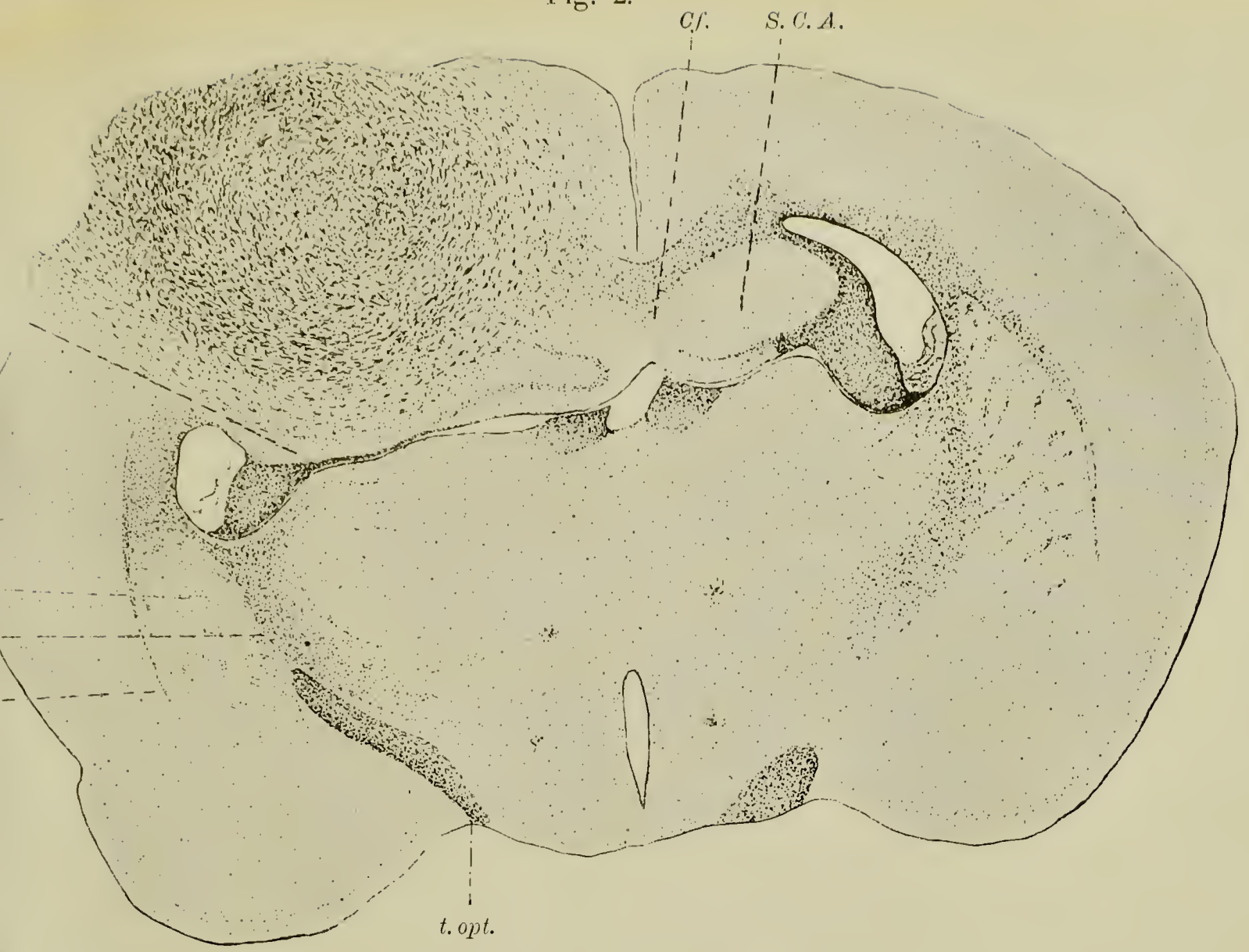
Fig. 2.



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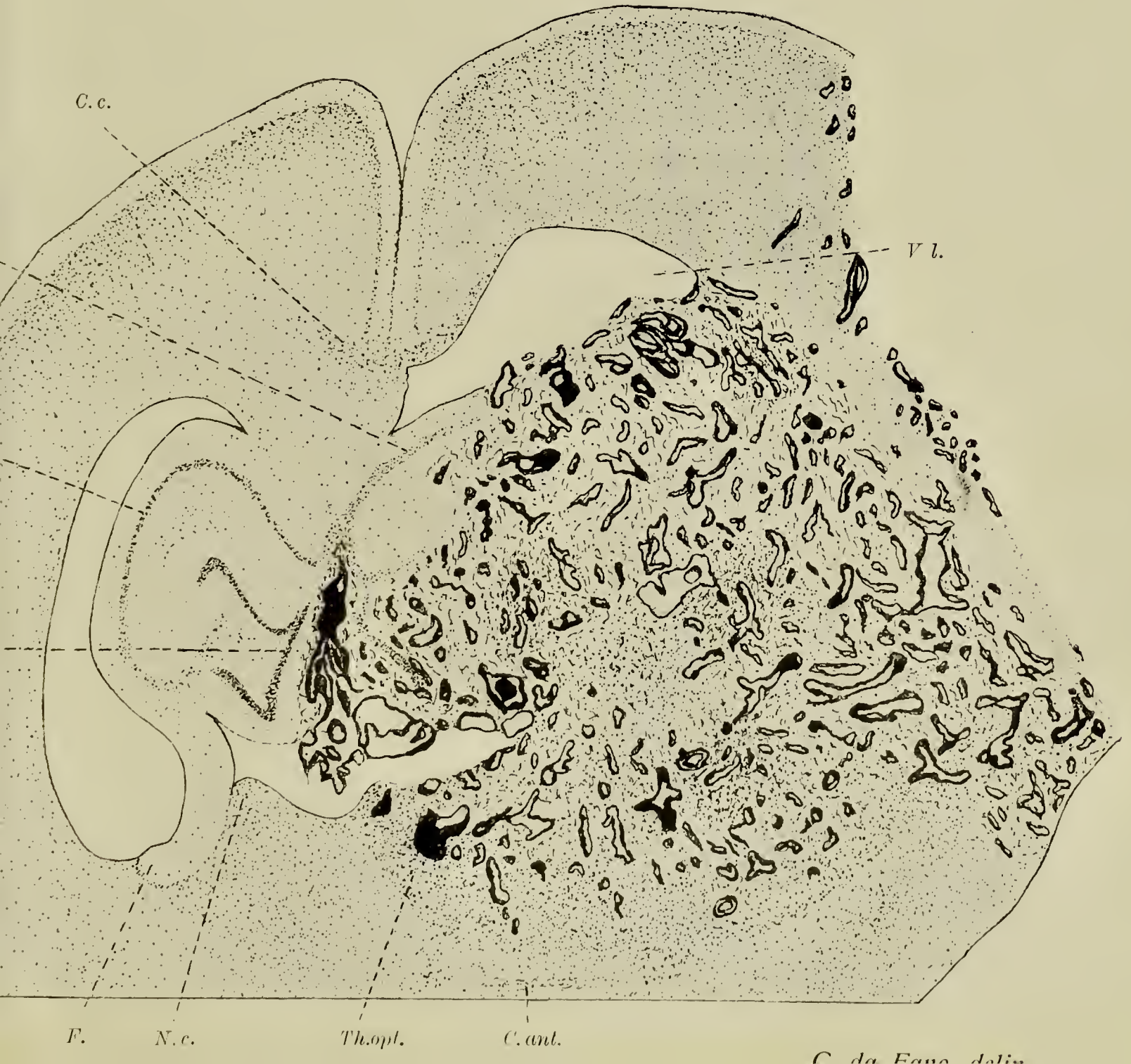

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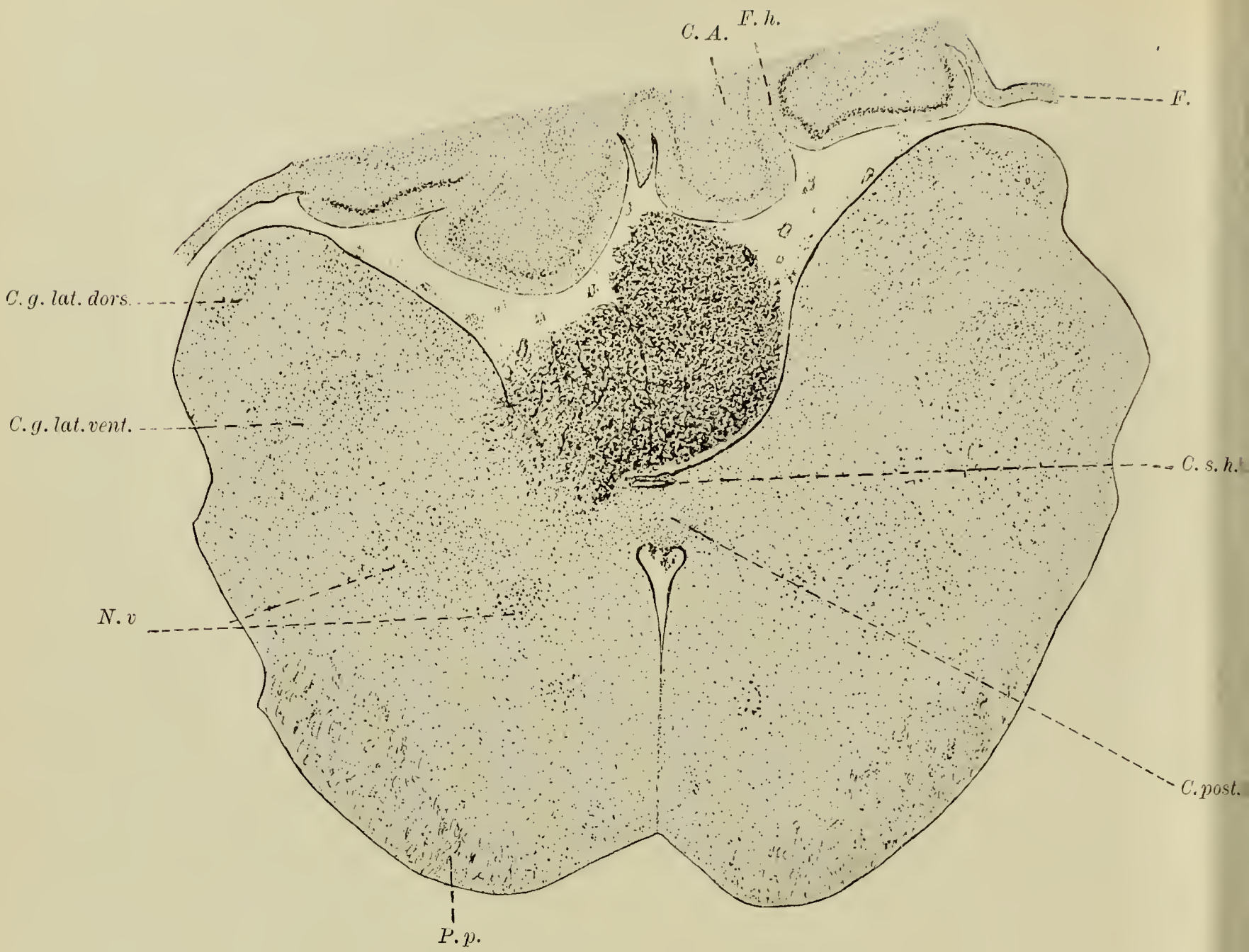
Fig. 5.



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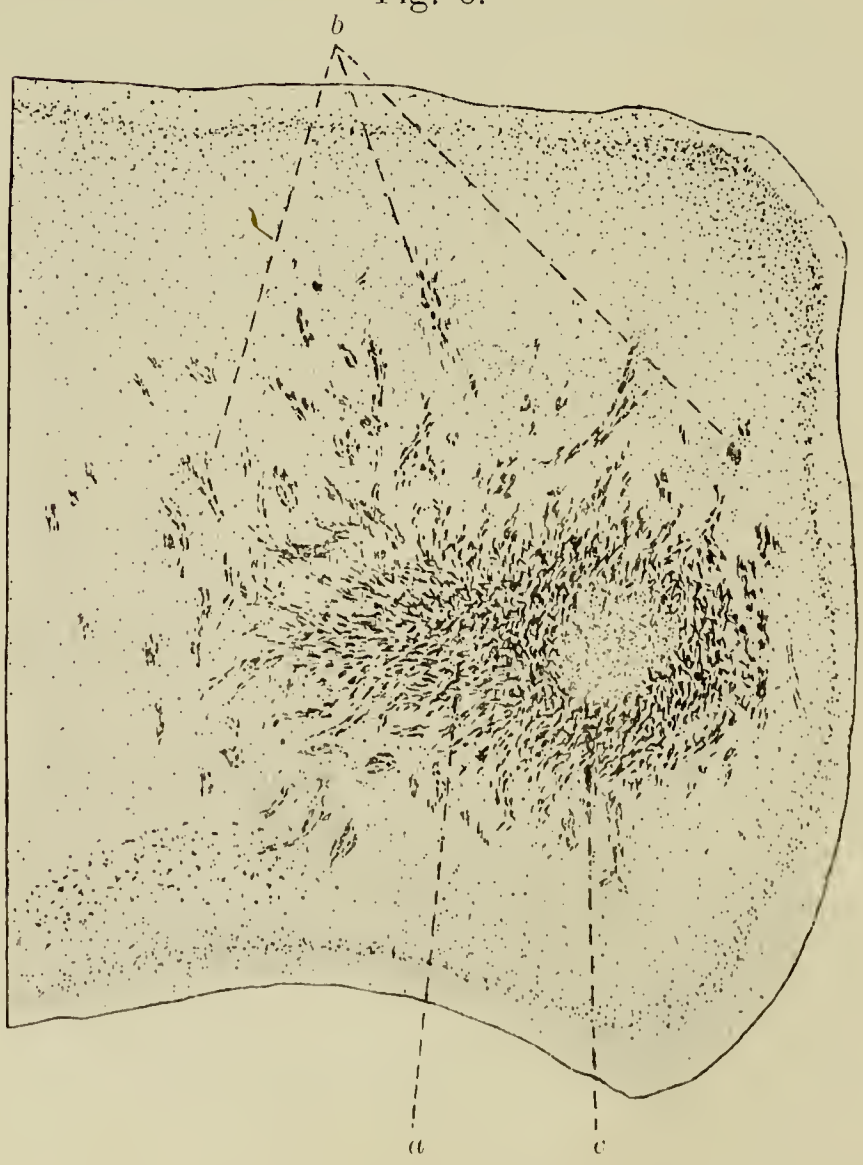

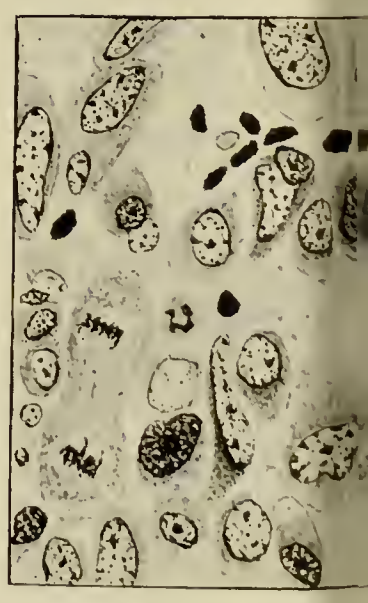

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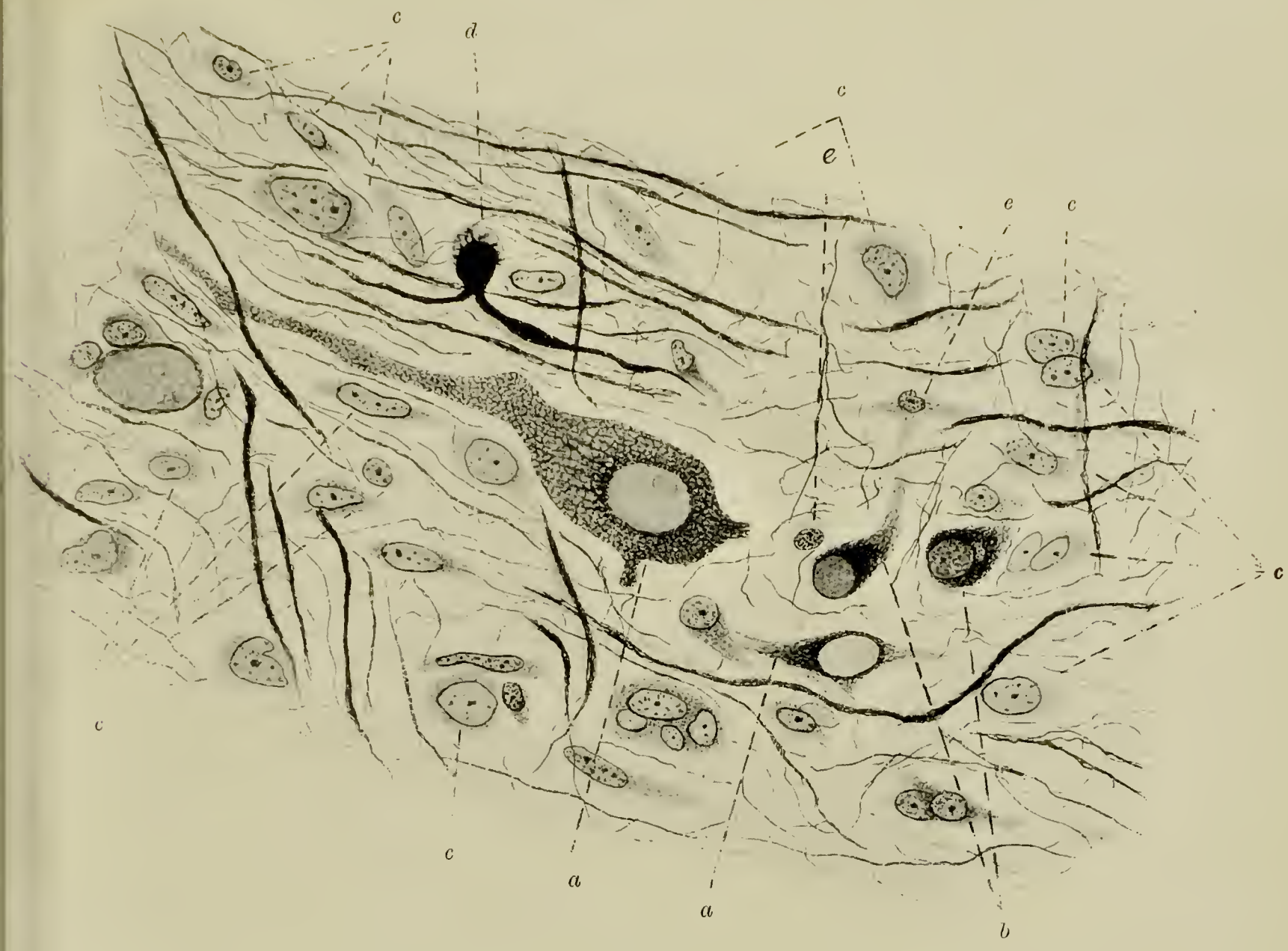

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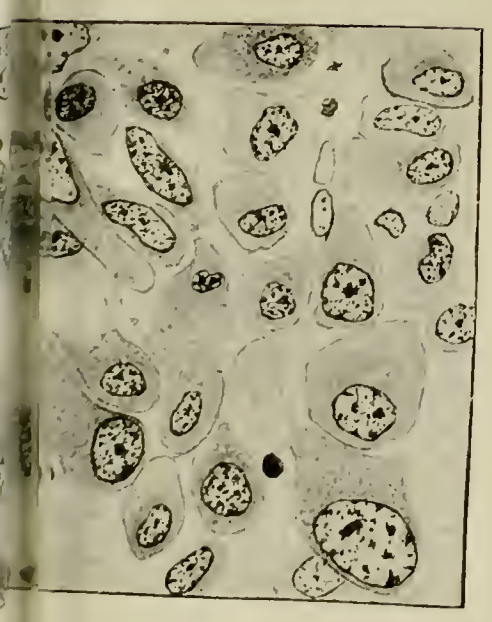

Fig. 9.

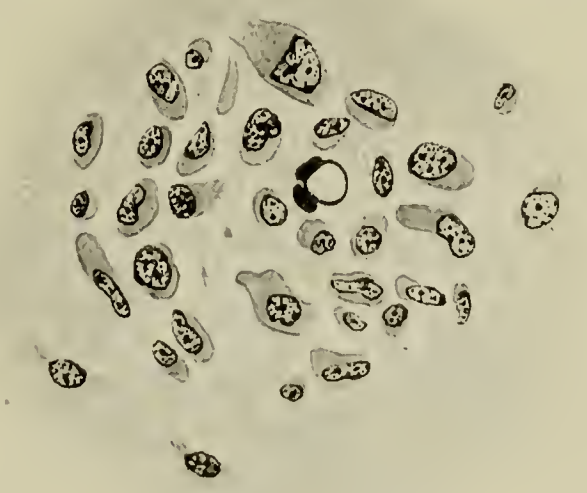

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Fig. 10.

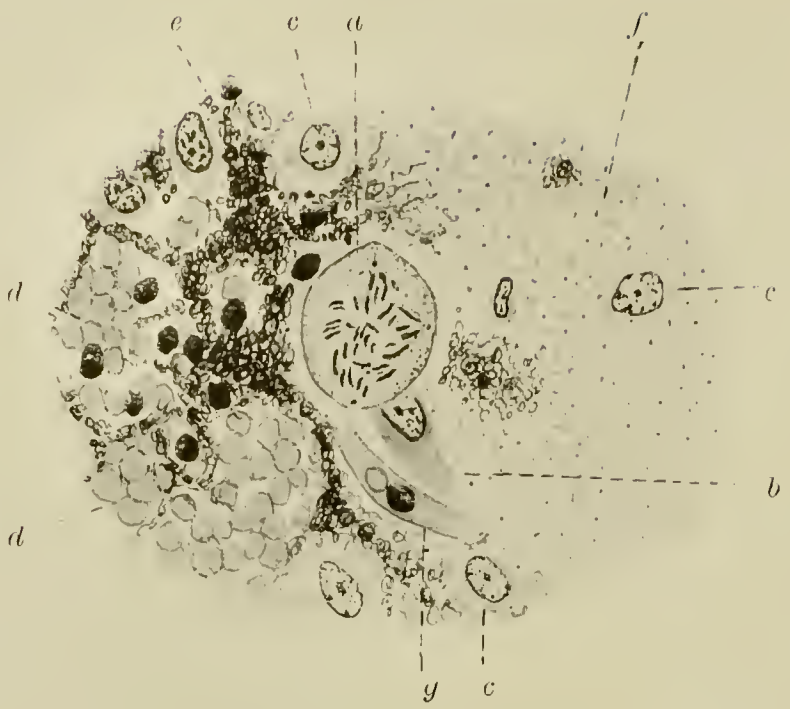

Fig. 13.

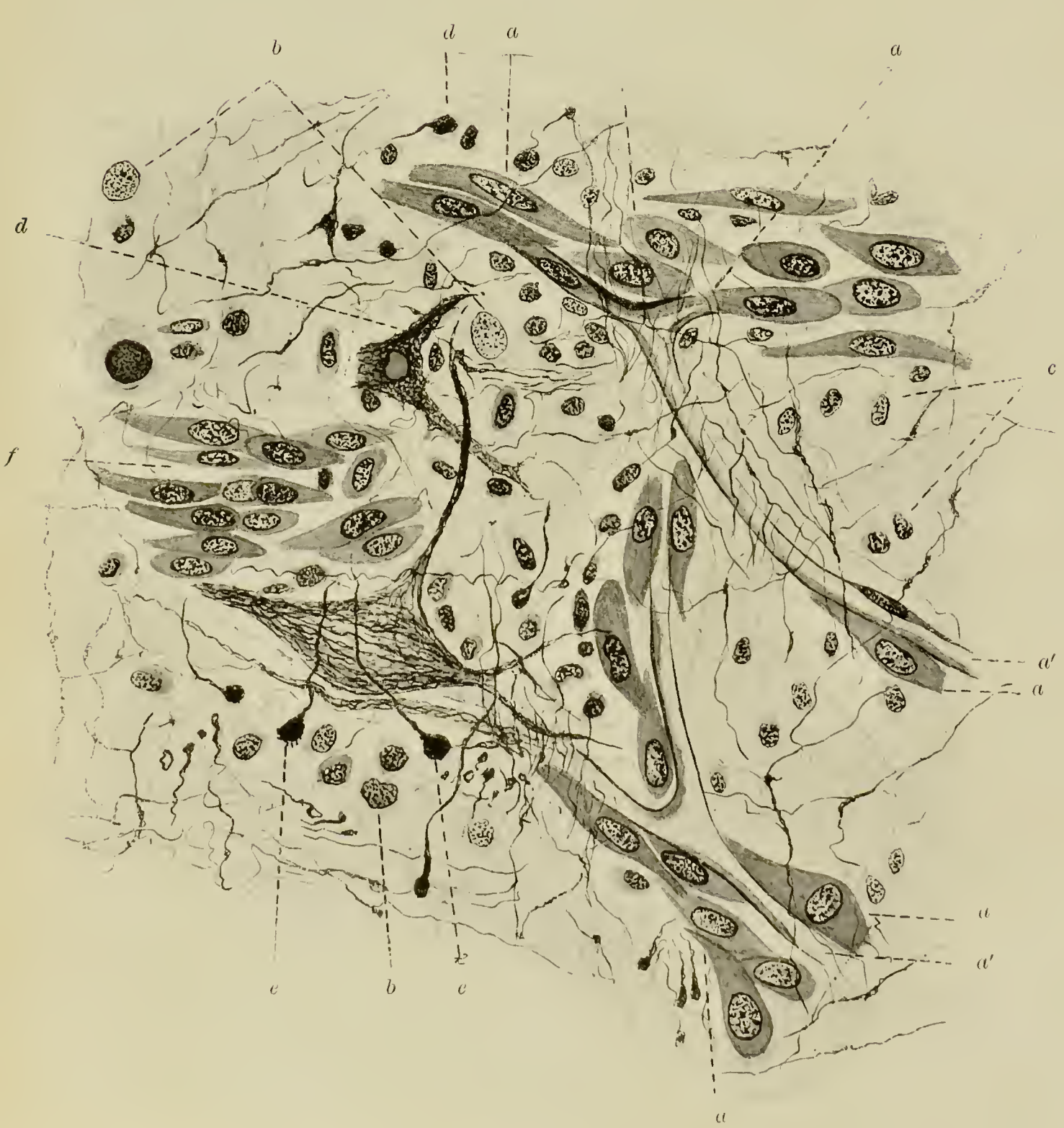

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Fig. 11.

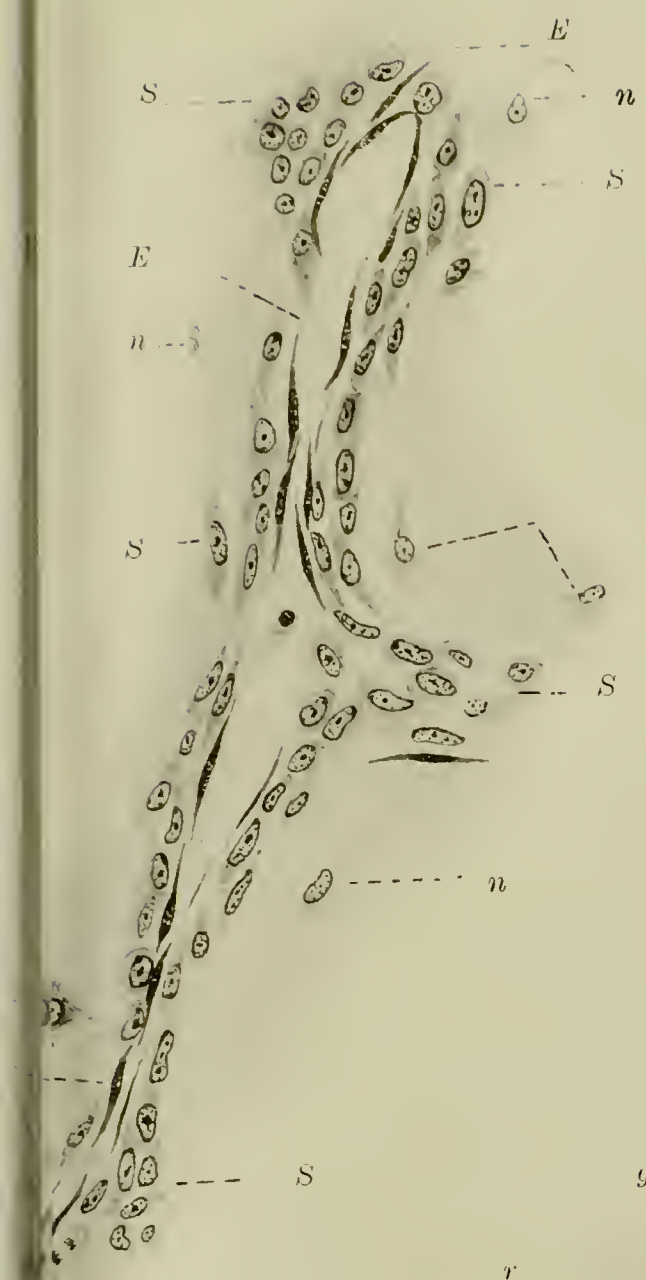

Fig. 12.

Fig. 14.

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Fig. 15.

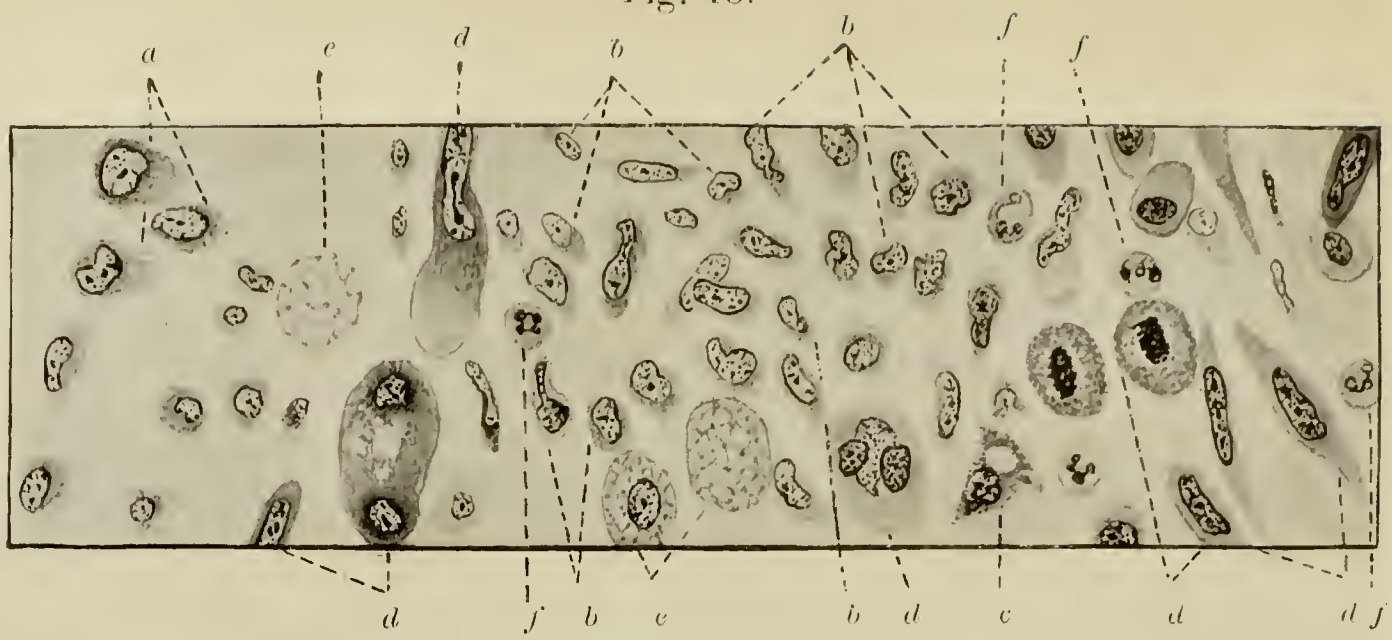

Fig. 19.

Fig. 16.

Fig. 17.

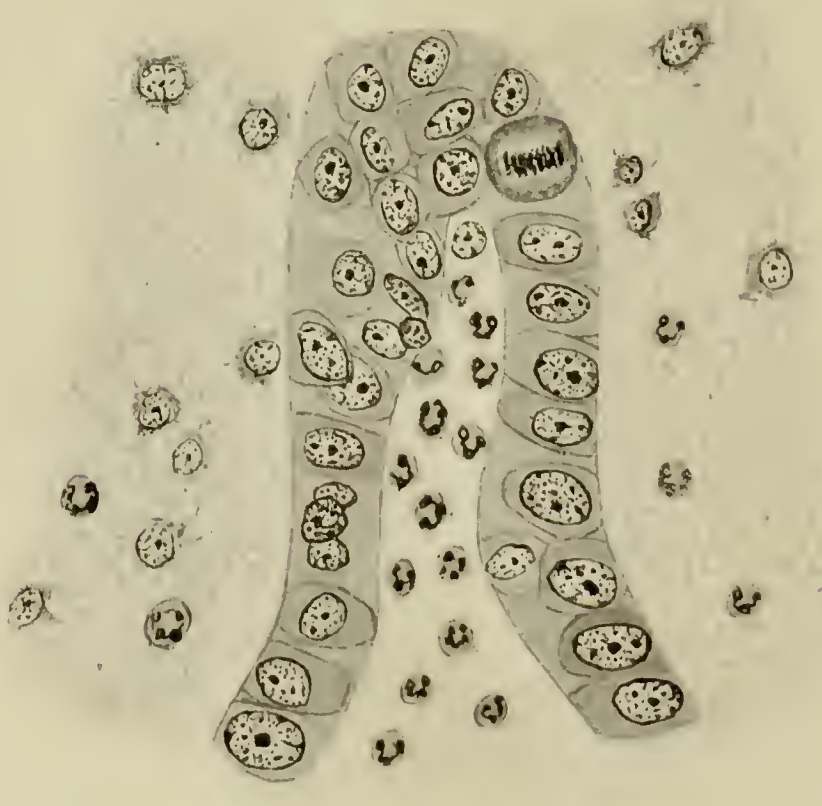

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Fig. 18.

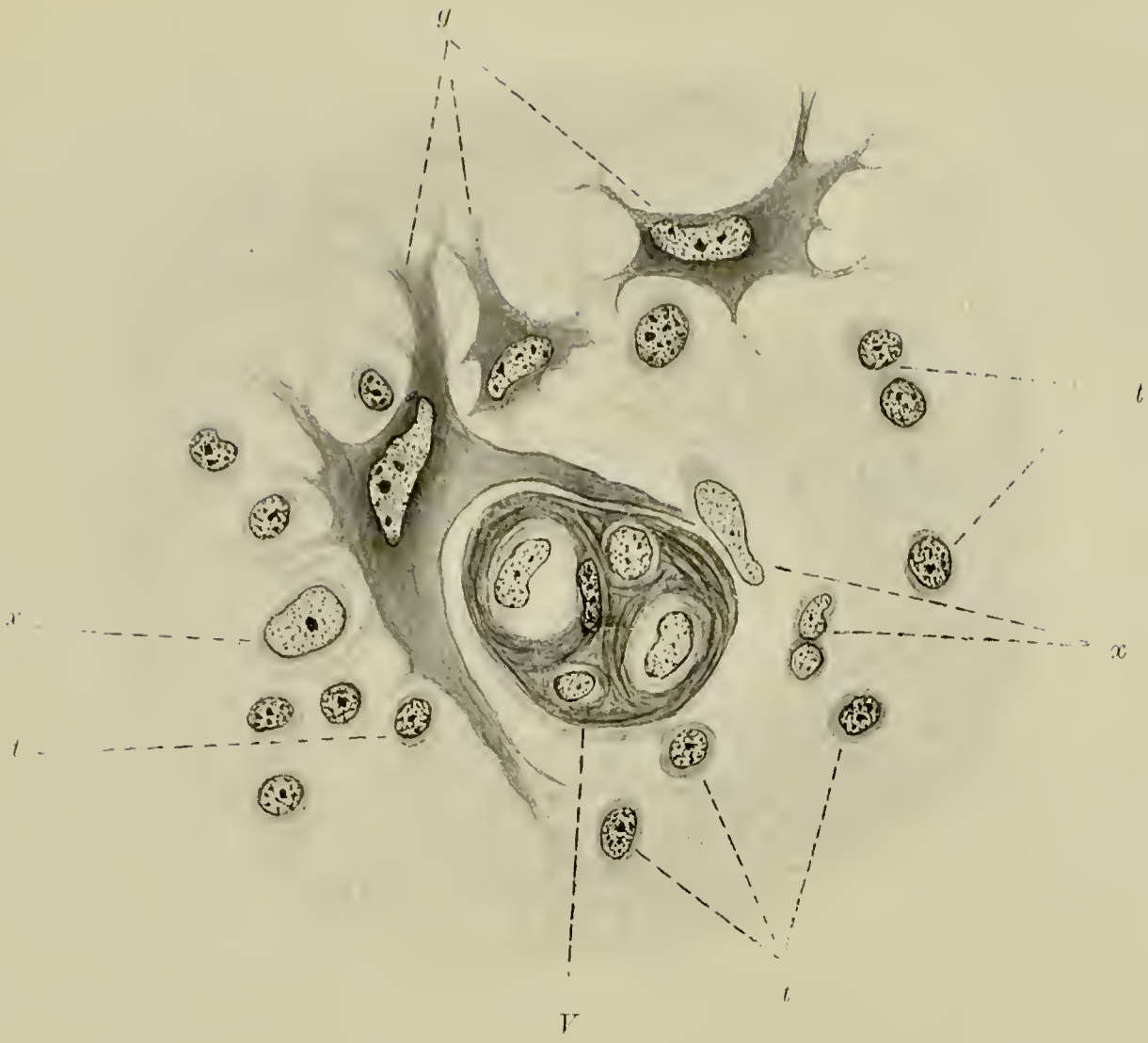

Fig. 20.

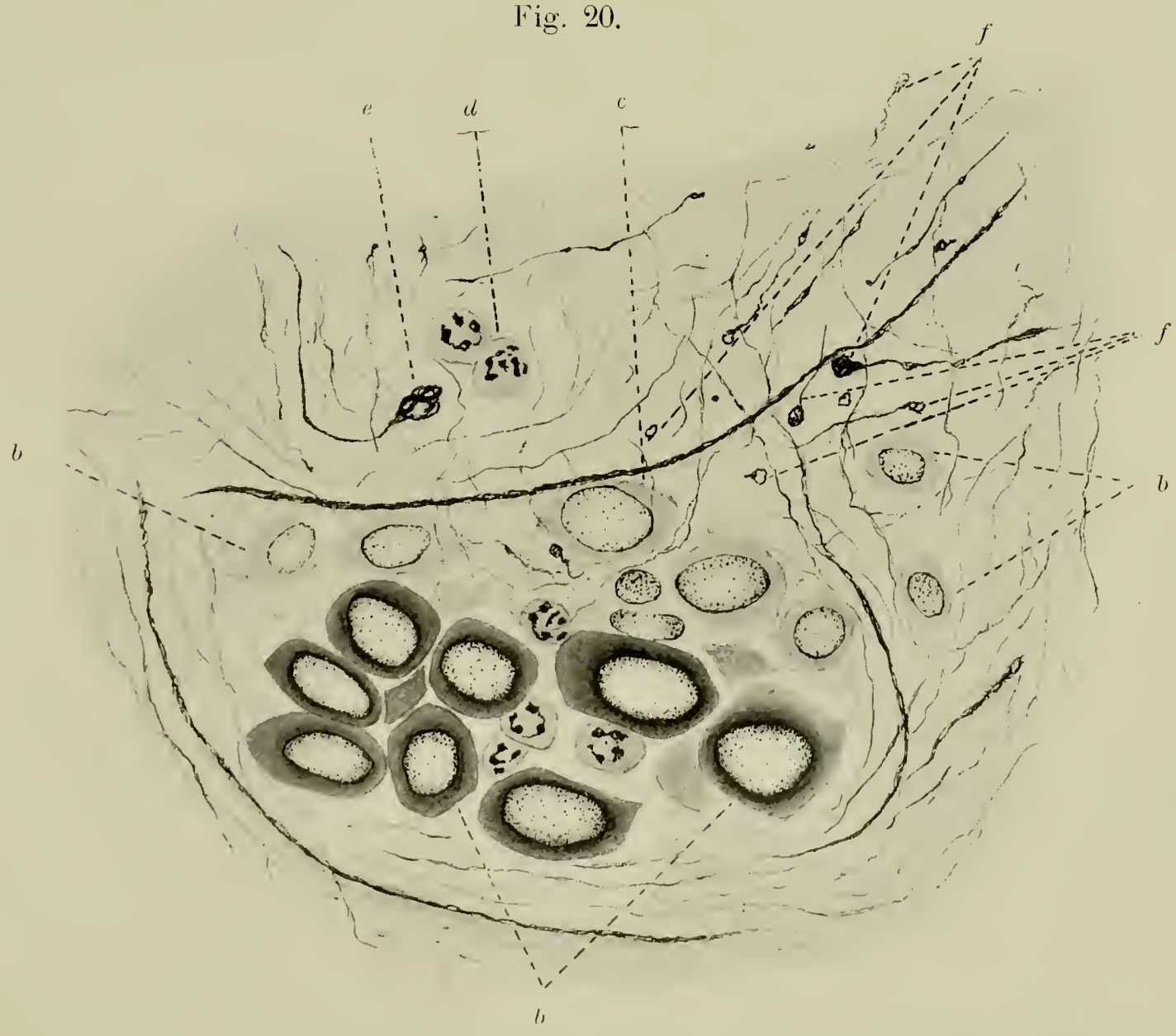

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Fig. 21.

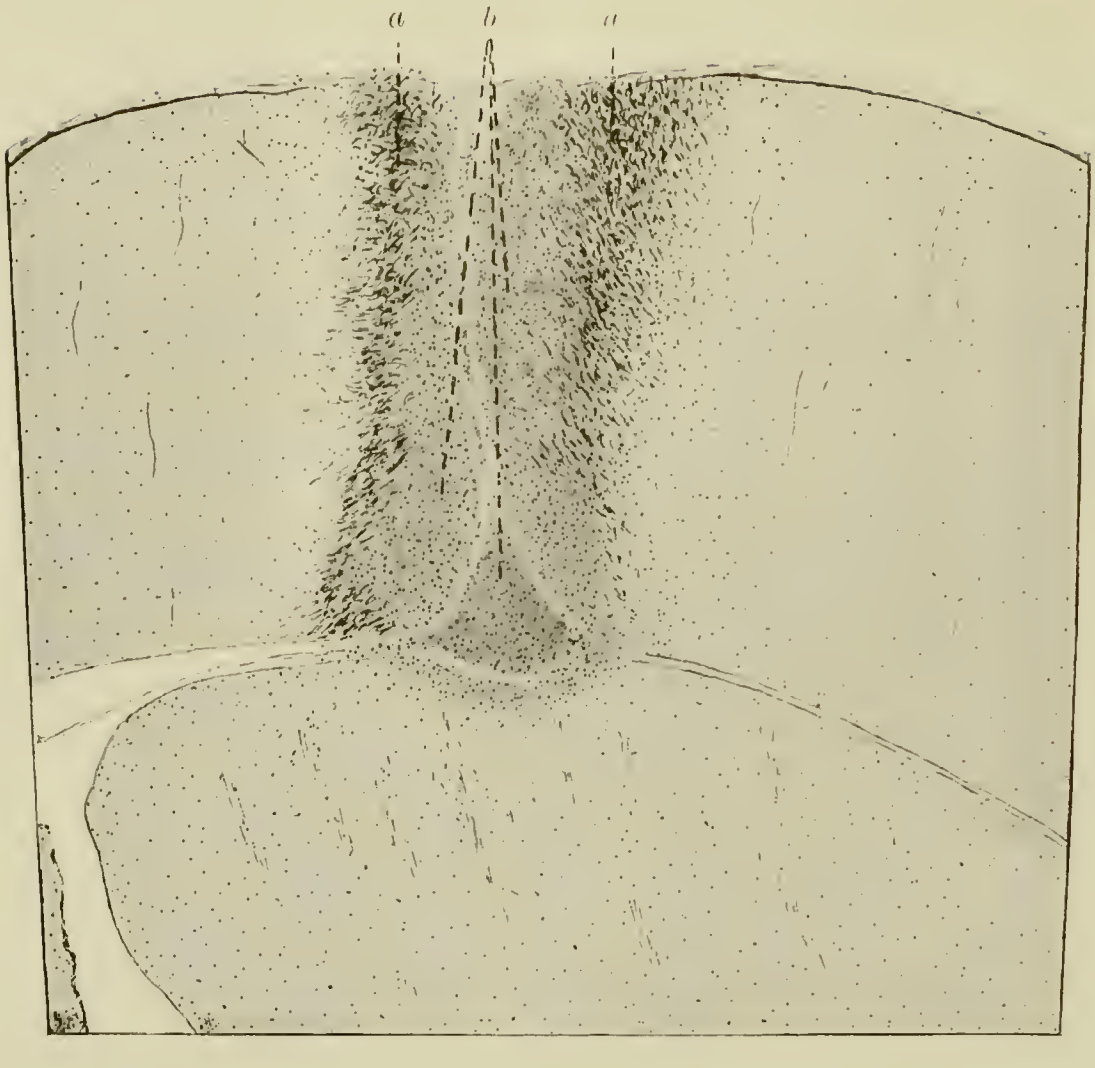

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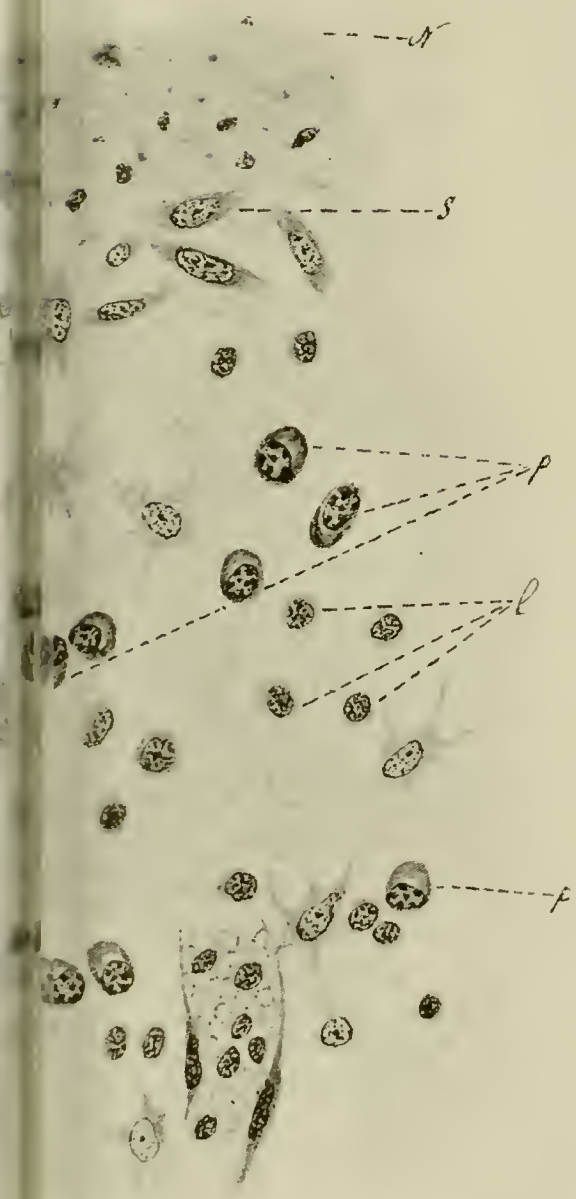
Fig. 22.



C. da Fano, delin.

Intracerebral Transplantation of Malignant New Growths.

by

C. Da Fano, M.D.

(From the Laboratories of the Imperial Cancer Research Fund.
Director — Dr. E. F. Bashford.)

(With five Plates).

The following investigations[1]) were made with the double purpose of establishing: — (1) whether malignant neoplasms of rats or mice could be transplanted successfully into the brain; (2) whether in this way the alterations caused by growing tumours in the nervous tissue could be systematically investigated experimentally. For this purpose human pathology has at its disposition only the occasional findings at autopsies and of some very few successful surgical operations.

The results of some preliminary investigations permit an affirmative answer to the first question: when carried out with proper technique, intracerebral transplantations of malignant new growths of rats or mice succeed with the same percentage although with a somewhat slower rate

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[1]) This investigation was carried out in London between February and July 1909 For reasons quite independent of my will this paper could not be prepared for publication sooner. It is incumbent on me here to express my most respectful thanks to the Committee of the Imperial Cancer Research Fund for the facilities afforded me- Above all I am indebted to the Director of the Laboratory, Dr. E. F. Bashford, for placing the material and opportunities for experimenting at my disposal, and for his aid, support and ever ready advice. I return my warmest thanks to him and the Assistants of the Institute, Dr. J. A. Murray, Dr. M. Haaland and Dr. B. R. G. Russell, who also most courteously helped me in my researches. — I am also very grateful to Prof. Th. Ziehen of Berlin who most courteously helped me in the difficult orientation of some cuts.

of growth than subcutaneous inoculations. As to the second point only a certain number of interesting details could be ascertained of which an account will be given. They have to be understood only as points of orientation for the further study of a question about which it is necessary to make many more and minute investigations with extremely careful technique and with a series of different transplantable tumours. It seems, however, useful to relate in a preliminary form what has been thus far established, reserving the further elaboration of the interesting argument to another publication.

I. General Considerations — Method of Propagation — Material.

The first attempts at intracerebral transplantation of malignant neoplasms were made on mice and were very unsuccessful; the little animals died either during the operation or some hours after in consequence of serious haemorrhages. New experiments however were successful. It was possible in this way to establish some technical rules which may be valuable also for the intracerebral propagation of tumours in larger animals, as e. g. in dogs, though with these the danger of immediate death after the operation is much less and the method of propagation may be altered at the will of the investigator.

Complete ether-anaesthesia is necessary. In the London Institute it was found convenient to put the animals in a glass jar on the bottom of which lies a small piece of cotton-wool on which some drops of ether have been poured. As soon as the animals sink down on the bottom of the jar, they must be taken out and can now be placed upon a very small operating table. Hereafter the anaesthesia is continued by blowing air through a bottle containing ether and conducting the ether-laden air to the animals' nostrils by a small funnel attached to the outlet tube of the ether bottle. Very few animals died in consequence of anaesthesia.

A second point is the necessity of perfect sterilization of the instrument, and of the aseptic removal of the tumour chosen for intracerebral propagation. For this second purpose I have found the method, already described in different papers from the laboratory, very practical and simple.

For intracerebral propagation, the only special instruments required, are a very small and sharp trephine and a hollow platina needle of small calibre with a tightly fitting plunger as have for some years been used at the Imperial Cancer Research Fund, but with a very short point.

The operation is carried out in the following way: the tumour, removed aseptically some minutes before starting the operation, was kept in a small sterile glass-box; the animal about to be operated on was quickly narcotized and placed on the small operating table; the skin of the head was carefully shaved, to prevent hair from getting into the wound, and then washed with alcohol and ½ per mille sublimate. Then a little incision of ½ cm. was made on the middle line and drawing back the skin to the right or to the left of

the middle line, there lay bare a small portion of the skull upon which the point of the trephine could be directly applied; with rats it is better also to remove the periosteum with the blunt side of the knife. The trepanning is a delicate procedure; it is necessary to trepan almost without pressing on the skull as even very slight pressure will suffice at once to kill a mouse. When the trephine has made a hole scarcely large enough to introduce the point of the needle it is better to withdraw it at once, and at the same time to let the skin go, which because of its elasticity covers the small hole.

The second part of the operation consists in cutting from the tumour, beforehand removed and prepared, a very small fragment, putting it into the hollow needle and introducing it intact under the skull. For this purpose it is necessary to draw aside the skin, slowly and prudently to push the point of the needle through the opening in such a way that the direction of the needle be not perpendicular to the surface of the skull but very slanting so that the point of the needle cannot penetrate into one of the lateral ventricles, which could kill the animal, especially if a mouse. By gradual pressure on the plunger the small graft is pushed into the cerebral substance and generally remains in the cortex of the brain. The needle is now withdrawn very slowly and the wound of the skin closed with one stitch.

The difficulties and dangers of the second part of the operation are different: it is first of all necessary to exert only a very gentle pressure on the skull with the needle for the same reason mentioned above with reference to the use of the trephine; secondly, it is necessary to prevent the needle from penetrating into one of the lateral ventricles, which is very difficult, as the cortex of the brain of mice and rats is very thin; thirdly, it is possible for the graft to be drawn back with the needle. In the latter eventuality there are two possibilities: either the small fragment of tumour comes out completely and the operation has been done in vain, or the fragment sticks in the dura mater. In the second case the tumour will grow as well as in the brain, but the tumour develops in the dura mater and turns out an extracerebral neoplasm. It does not penetrate into the cerebral substance but forms therein a depression, from which it can easily be taken out.

In some experiments tumours, of the dura mater (sarcomata) were obtained unintentionally, owing to the above described technicality, and they behaved in the same way as human tumours of the meninges with development in two directions outward into the skull, inward into the brain. These observations are but casual, but they show that grafts of sarcomata can grow outwards towards the dura mater and giving origin to extracerebral tumours compress the cerebral substance itself, without infiltrating it. Their importance consists not only in the fact that they may indicate a method of systematically studying the consequences of gradual compression of the cerebral substance by extracerebral tumours, but also in the fact that a graft of the same tumour (rat sarcoma) can

grow in a slightly different way, according as it has been inoculated into the dura mater or into the cerebral parenchyma. When growing into the dura mater, the tumours appear as compact nodules with very scanty and delicate stroma made of fine blood vessels and capillaries; these nodules grow expansively. The intracerebral tumours on the contrary, as shown in the following pages, grow infiltratively, following the direction and division of the bloodvessels.

To overcome the difficulty due to the small grafts coming back with the point of the needle through the opening in the skull, an emulsion of the tumour in normal saline solution was injected. In this way it appeared also possible to obtain many small tumours in the same brain and to be able to study more easily the minute details of their growth. Two such experiments were made, but the result of the microscopical examination of the two brains led me to desist from such attempts for the present, because the brains had to be cut almost entirely in serial sections, which in preliminary researches was not practical; and also because the features of all these little tumours growing up in the same brain were complicated by haemorrhages and by reactive phenomena on the part of the blood vessels. All this increased the difficulty of an exact analysis of the way in which the tumours grew and the result of the experiment was at last rather complicated than simplified.

For the further examination of the growing tumours, the animals may be killed at will. It is impossible to know beforehand whether the tumours have grown and how large they have got. The growth of subcutaneous grafts of the same tumour in other animals may serve to fix approximately the time of killing, if the fact be allowed for that intracerebral tumours grow a little more slowly than ordinary subcutaneous grafts, i.e. that when compared with subcutaneous grafts of the same age they are a little smaller. This seems to find a natural explanation in the difference of the initial doses. For the intracerebral inoculations very small doses (½ mmg.) were used, whereas for the subcutaneous inoculations the dose used was at least ten times as large. Since accurate control researches were not made with equal doses of the same tumour on animals of the same age, too great a value is not attached to this interpretation. It would however be useful to make some experiments of this kind, as the blood supply of intracerebral grafts in the different periods of their growth might so be more carefully studied.

The intracerebral grafts seem quickly to be vascularized when in their first stages and to be in a good nutritive condition; necrosis of central parts, even in comparatively well developed neoplasms, is very rare. In grafts of rat sarcoma, e.g., small necrotic areas were found only during the first 3—5 days, whereas they were not to be found in the same tumour in the later stages till 11 days after inoculation. With mouse carcinoma "T", necrotic areas were never noticed. We might con-

clude herefrom that the nutritive conditions for the same tumours are better in the brain than in the subcutaneous tissue, which again may be explained by the great richness of blood vessels of the brain. Hence it is somewhat incomprehensible why the intracerebrally inoculated tumour should grow more slowly than the subcutaneous grafts. Tumours that are better nourished should also grow relatively more quickly even when the inoculated dose was smaller. In the slower growth of intracerebral grafts there are probably other factors playing a part therefore, of which as yet very little is known and which might be ascertained by accurate control-experiments.

Another rather interesting fact may be mentioned, viz. the nearly complete absence of symptoms of the growth of tumours transplanted into the brain. Not till the tumours have reached a size which, compared with the minute proportions of the brain of a mouse or rat, is really enormous, is it possible to notice respiratory disturbances, characterized by frequent and very short respiration occurring a few hours previous to the animal's death, and only when the breathing of the animals became very bad, were phenomena of paresis of one or two members on one side exhibited, but never undoubted symptoms of paralysis. Tumours of about the size of those represented in Plate III Figs. 1 and 2 did not cause any noticeable disturbance, nor did they do so when situated in the cerebral cortex. No symptoms were called forth by the tumour represented in Plate III Fig. 3 though it had destroyed a great part of the brain cortex and the underlying medullary substance, between the frontal and occipital poles which themselves were not altogether unimpaired. Severe respiratory disturbances however were observed in the rat, the tumour of which is represented in Plate III Fig. 4; the destruction of cerebral substance in this case was very extensive; the tumour though under the cortex reached the oral and nearly the caudal extremity of the right hemisphere; where it had its largest size, it had destroyed part of the cortex, the medullary substance, the cornu Ammonis, the optic thalamus, the corpus striatum of the right side and also part of the optic thalamus of the other hemisphere. Respiratory disturbances appeared however nearly two months after the transplantation and when growth of the tumour through the skull had already been evident for 15 days previous; the breathing disturbances were observed in the morning and the animal was not killed till the afternoon, 6—7 hours later, and then only lest the rat might die during the night. Before being killed the rat could, if stimulated, still run across a large table; in this way it was possible to remark that, while running, the respiratory disturbances increased and that the posterior left member was paretic but not completely paralysed. To give a rational explanation of such pheno-

mena without having recourse to useless speculations seems at present impossible.

For the experiments about 40 rats and 20 mice were used. The inoculations were made with a spindle celled sarcoma (J. R. S.) and with a carcinoma (F. R. C.); in the case of mice, carcinoma „T" was inoculated. As the growth of the intracerebral grafts of the three tumours all appear in a certain way different, it seems useful for greater clearness in the following pages to expose the results of the three groups of investigations separately.

For the histological examination of the intracerebral grafts the usual methods of fixing and staining were adopted and some other methods more appropriate to the investigation of the nervous tissue, e. g. the methods of Nissl, Cajal and Bielschowsky and the methods proposed by Benda and by me for the staining of the neuroglia. Very good results were obtained with fixation in alcohol and subsequent staining with Azur II (Grubler's solution) as already described in my paper „Zelluläre Analyse der Geschwulstimmunitätsreaktionen."

Of a special group of researches made on immune animals a short account will be given in the last chapter of this paper.

At the end of these general considerations mention may be made of attempts at intracerebral propagation of the venereal dog tumour propagated in the London Institute. The inoculation of this tumour into the brain of dogs was, as is well known, demonstrated by Sticker, who however did not investigate the early phenomena following inoculation. This tumour was used, on the one hand, merely in order to demonstrate that the technical rules described for the propagation of malignant new growths into the brain of rats and mice, are also suitable for bigger animals: on the other hand, to prepare an object of comparison with the intracerebral grafts of malignant new growths in rats and mice. It was also desired to prove that with a very simple technique and hardly any loss of blood it is possible to cause a tumour to grow in any area of the brain of dogs and to study in that way the effects of the slow and progressive destruction of certain portions of the brain. Such a method might be employed in many experimental researches for which till now hardly any other system was used than that of surgical removal of part of the brain.

Preliminary observations show that, in principle, the thing is possible: with hollow needles of different lengths it is possible to transplant small portions of this tumour into different places of the brain of dogs, where they develop into tumours. A tumour developed in the third ventricle after intentional inoculation in that site with a long needle and the operation did not in any way hurt the animal. The needle had gone straight across the ventricle and had slightly touched the surface of the left optic thalamus where the little graft stuck. Unfortunately the animal was killed too soon and so the opportunity of studying the fur-

ther consequences was lost. Yet the little tumour has, as we shall craftwards see, been useful as material for comparison.

II. Intracerebral Propagation of Rat Sarcoma. („J. R. S.")

For this first group of researches 20 young rats were employed. They were killed 3, 4, 5, 10 and 17 days after the operation; every time four rats were killed so as to have material enough for different methods of fixation and of staining. The result was positive in all the animals; in the rats killed 3, 4, 5 and 10 days after the propagation, each four tumours of the same age had reached about the same size. In only two of the four rats killed after 17 days were tumours found of the size represented diagrammatically in Fig. 3. In two other animals the tumours were about half that size. In none of the 20 animals was it possible to notice definite symptoms of the growth of intracerebral tumours.

In the brain of the rats killed 3—5 days after the operation the first fact attracting attention was the peculiar aspect of the little tumour in preparations examined with slight magnification. Plate IV Fig. 6 gives a sufficiently exact idea of this special configuration: a central nodule, corresponding to the point where the graft first penetrated, with large cells, easily recognised as sarcomatous elements, and limited necrotic areas; around the central nodule a great number of secondary and very small nodules scattered in the cerebral substance also at a considerable distance from the site of implantation. Plate IV Fig. 6 was drawn from a section of the tumour where its diameter was greatest; following the whole length of the tumour in frontal serial sections it was easy to remark that the radial configuration of the secondary nodules reappeared throughout, only the general form and the position of the different nodules changed a little with respect to the central mass; in the terminal oral and caudal sections the central mass disappeared whilst the secondary knobs still penetrated a remarkably long way into the nervous tissue. In other words the growing tumour does not at any point appear to be distinctly separated from the surrounding cerebral substance, which was on the contrary completely infiltrated with sarcomatous cells.

In the larger-sized tumours the central mass had greater dimensions; at its periphery however there still existed a large zone of infiltration occupied by secondary nodules and small groups of sarcomatous elements (Plate III Fig. 3). Because of the larger size of the whole tumour and the greater practical difficulty therefore of cutting it in complete series, the phenomenon was not so evident. The zone of infiltration did not exist except in one case, (Plate III Fig. 2), where the tumour grew in part extracerebrally; in this case however there did not exist a distinct limit between tumour and nervous tissue, but the sarcoma passed gradually into the cerebral substance in such a way that at the periphery of the clearly

noticeable tumour parenchyma, there existed a zone of doubtful nature. We shall see afterwards of what elements this latter zone consisted.

It is necessary to insist somewhat on this point because the infiltrative growth of the sarcoma transplanted into the brain of rats contrasts with what we learn from human pathology; really primary sarcomata of the nervous tissue of man generally form tumours with expansive growth, which can relatively easily be enucleated and removed from the surrounding tissue. In human pathology however cases are known of sarcomata of the central nervous system with infiltrative growth; such tumours correspond with those produced through transplantation in animals. One of such cases is at present under my observation, and I reserve a detailed account of it to another paper. Plate IV Fig. 7 shows a place however where the sarcomatous elements and their infiltrative growth are very evident. It demonstrates very clearly that sarcomata of the human brain in some cases give pictures nearly indentical with those experimentally produced by grafting a transplantable sarcoma into the brain of a rat.

The examination of the preparations from the early stages of intracerebral grafts of sarcoma, under greater magnification, brought to light other peculiarities. In the first place the strongly pronounced polymorphism of part of the small neoplasm. Whereas in some parts of the graft the typical spindle form of the cells of the sarcoma was perfectly retained, in other places this original form was hardly recognisable, and had in others completely disappeared. Some cells were again spindle-shaped but unusually large; others on the contrary were reduced to very small proportions. Between these cells, either isolated or lying in groups, were other sarcomatous elements, roundish, oval, irregularly quadrangular, of different sizes, very often with a big nucleus also very irregularly shaped, or multinucleated. Some of these cells were in different stages of mitotic division. The Figs. 8 and 9 Plate IV give a sufficiently clear idea of this phenomenon; the first was drawn from a part where large-sized elements predominated and where it was again possible to recognise some spindle cells; the other illustrates better the appearance of groups of very small elements, which however do not show any alteration suggesting real atrophy; they have to be looked upon as small but unaltered cells. The smaller forms prevailed in the above mentioned secondary nodules, whereas the larger types were more numerous in the periphery and also, in some preparations, in the middle of the principal mass. In the following stages, when the grafts had grown, isolated examples or little groups of irregularly shaped elements were again present, but the original spindle-aspect of the great majority of the sarcomatous cells predominated.

The difficulty of a rational explanation of this phenomenon is augmented by the almost complete disappearance of real polymorphism in the larger-sized tumours. If the polymorphism had been constant the phenomenon would be clear enough. We know indeed that the aspect of the elements of a tumour may change during propagation from one animal to another of the same species, or in consequence of the exposure of the cells of the tumour to a physical agent insufficient to kill their growing power. The cellular polymorphism appeared in the intracerebral grafts as a transitory feature in the early stages, but it was impossible to follow its disappearance, since the different stages of the tumours were not investigated regularly, from one day to another. It is therefore impossible to say when the phenomenon disappeared and whether gradually or suddenly. Any explanation will therefore somewhat bear the character of an hypothesis.

Considering the general results of recent experimental studies about cancer, especially of the researches carried on in the London laboratories, the most rational supposition consists in putting the phenomena of cellular polymorphism in connection with the altered conditions of existence. The brain represents for the transplanted cells a new environment to which they have to adapt themselves; the appearance of many differently shaped and differently sized elements in the first days after transplantation are here to be understood as the morphological expression of an effort at adaptation to the new environment. As, however, the alteration in the conditions of existence was very small and certainly not to be compared with the alterations following on the initial transference of a malignant new growth from the spontaneously affected animal to the first series of normal animals, the adaptation manifests itself very quickly and already 10 days after the transplantation the cells reappear in their original morphological aspect whilst the atypically shaped cells have disappeared. Hereby some light would be thrown on the slower growth of intracerebral grafts of certain tumours and on the occurence of necrotic areas, especially in the first days after propagation, as these two phenomena may also be understood as the result of some difficulty in the adaptation of the cells to the new conditions of existence.

It is to be added here that a certain tendency to polymorphism may already have been innate in the cells of the rat sarcoma. In fact, when I made my experiments of intracerebral propagation, „J. R. S." was a spindle cell sarcoma; but from a personal communication of Dr. Murray's I have learnt that at present this rat sarcoma is growing as a polymorphic-cell sarcoma. It may be supposed that the intracerebral propagation, i. e. the somewhat altered condition of existence has given rise to the temporary and anticipated occurence of a phenomenon which at a later period might appear permanent.

The above-mentioned phenomenon of cellular polymorphism has made it possible to establish with some certainty the nature of a few larger elements in different phases of mitosis and observed in the cerebral tissue, generally at a relatively great distance from the site of implantation. As represented in Plate V Fig. 10 we have to do with large roundish cells, which in comparison with doubtlessly sarcomatous elements, have very thin and delicate protoplasm. The presence of these cells in the earliest stages only, their situation in the scarcely altered nervous parenchyma and the somewhat different aspect of the protoplasm, raised doubts at first about their nature, so that one was inclined to take the above-mentioned elements for enlarged cells of the adventitia of the blood-vessels. In fact they were always situated in the immediate neighbourhood of blood vessels. The atypic mitosis however to a certain degree contradicted this. The phenomena of polymorphism afterwards observed and the study of these elements in serial sections favour their being somewhat atypical sarcomatous elements, grown out in the nervous tissue and representing the most advanced cells of an infiltratively growing tumour. The situation of the large cells in the immediate neighbourhood of blood capillaries or blood vessels did not contradict the second supposition, as in the intracerebral grafts of rat sarcoma, the infiltration of the nervous tissue seems nearly always to proceed along the blood vessels.

This last fact has to be considered here more carefully, as the intimate connection existing between the growth of the sarcoma in the brain of rats and the blood supply cannot be without interest. (Plates IV, V Fig. 9—11—12). The phenomenon was most evident in the earlier stages. Studying under high magnification the above-mentioned secondary nodules surrounding the central mass in 3—5 days old grafts, every nodule was seen to consist of a certain number of sarcomatous elements grouped in different ways around one or two blood capillaries (Plate IV Fig. 9). In some cases, especially in the smaller nodules, the phenomenon appeared almost schematically (Plate V Fig. 12). Some cells were gathered in groups along dilated capillaries Plate V (Fig. 11) or infiltrated into the perivascular lymphatic space so as to give the same picture — apart from the nature and form of the elements — of perivascular infiltrations in general paralysis. Often the small number of sarcomatous cells lay between many capillaries, the walls of which were formed of a few endothelial cells (Plate V Fig. 12). Special attention was drawn to similar nodules as they may prove that the sarcomatous cells in their progressive growth not only followed the general disposition of the blood capillaries already existing, but that the phenomenon was accompanied by an active formation of new capillaries destined to build a delicate stroma for the growing tumour. That part of the blood-vessels, around or in the neighbourhood of which the cells

were accumulated, were preexisting in the nervous tissue, is not to be doubted; some of the capillaries themselves however were certainly newly formed. In fact, studying the tissue surrounding the grafts, in those parts also where no sarcomatous cells existed, it was easy to follow the proliferation of the endothelial cells and the formation of new capillaries in the well-known way.

If the early stages were useful for the investigation of the above-mentioned details, the more developed tumours have proved especially suitable for the study of the alteration of the nervous tissue in which they grow.

As to the nervous elements, it seems convenient in this preliminary account to mention only two points. The very slow disappearance of the nervous cells from areas in direct connection with growing tumours, and the great resistance of the intracellular net-work of the nervous cells themselves is remarkable. In preparations stained according to Nissl's method or with Azur II, in the zones of nervous tissue quite near to the sarcomatous elements, there occur with a certain constancy many nervous cells the chromatin substance of which has lost its staining power and been dissolved. These cells appear in no other way altered and may be found in the parenchyma immediately surrounding the smaller as well as the larger sized tumours. Although the intracerebral grafts were first examined only three days after the inoculation, it may be taken for granted that the above-mentioned alteration manifests itself very quickly and is quite pronounced already on the third day. Cells more altered are to be found on about the fifth day and more easily in the following stages, but nearer to the sarcomatous elements. Here are to be seen nervous cells which are quite shrivelled up; the nucleus is also smaller; the nuclear membrane shows folds, and occasionally pyknotic changes. The most profoundly altered nervous cells have nearly lost their typical aspect and appear as roundish or irregularly shaped forms; their nuclei are small, pale, indistinct; it is clear that such cells are about to disappear. As the intracerebral tumours were not examined regularly from one day to another, it cannot be stated with great certainty how the different alterations have succeeded each other. The shrivelling up of the cells is probably secondary to the dissolution of the chromatin substance; finally there takes place a slow strophy of the whole cell. In the very early stages the dissolution of the chromatin substance probably represents the only alteration of the nervous cells; afterwards the altered cells become atrophic and disappear, while in the surrounding tissue other cells gradually lose or seem to lose their chromatic substance.

The preparations stained according to Bielschowsky's method have proved well fitted for the study of the alterations of the nervous parenchyma in the proximity of the sarcomatous grafts. In such preparations, moreover, the manner of the growth of the rat sarcoma in the brain shows itself most clearly. At the periphery of the tumours and also to a notable extent within it, where according to other methods sarcomatous cells only appear, Bielschowsky's silver impregnation revealed the presence of a great number of nervous fibrils interlacing in every direction. In between lay the sarcomatous cells, alone or in groups, very often arranged along the capillaries; in this way complicated pictures occurred, difficult to analyse, in which new blood capillaries, sarcomatous cells and fibrils lay side by side or covering each other (Plate V Fig. 13). Between these elements differing in nature and origin were to be seen, in several places, nervous cells, which also lay alone or in groups of two and three. It is especially noticeable that the greater part of these cells had kept a fibrillar net-work which could still be well stained. The careful examination of many preparations under high magnification showed several alterations, from a slight rarification to an almost complete atrophy of the fibrils. In some cells the alteration was only just noticeable as an undulating appearance of the fibrils; in others the intracellular net-work was formed of exceedingly small meshes or so changed in its arrangement that in some parts the meshes were large and regular, in others however very small. In cells that had changed more, the fibrillar apparatus appeared gradually to vanish; the protoplasmic prolongations were shorter and rarer, the nucleus easy to stain and slightly shrivelled. In still further altered cells there was nothing left of the fibrillar apparatus but isolated fibrils, or fibrils clinging together. In a word a complex of alterations which is not essentially different from those which several authors and myself have noticed in different pathological conditions of the human brain and in experimentally produced alterations.

Nerve cells have hardly ever been found quite without fibrils but with the original form and the characteristic arrangement of their protoplasmic prolongations as well as the situation of the nucleus remaining unchanged. In the few cases in which it did now and then occur, a careful examination or control-preparation made it possible to recognise the alteration as due to an error of technique. As in other pathological processes the fibrils disappear, but only in cells the atrophy of which has advanced to the last stage and where they have themselves already nearly disappeared. A breaking of the fibrils into small spieces and granules such as occurs, e.g. in cases of general paralysis, has never been noticed.

For comparison with the above-mentioned alteration some sections

of the sarcoma of the pons were treated according to Bielschowsky's method and preparations obtained in which it was clearly seen how the sarcomatous and nervous cells lay side by side and covering each other. When the pictures 7 and 13 (Plates IV, V) are compared it is striking how like they are. Only in the case of the sarcoma of the pons Varolii do the nerve cells, in consequence of their size, appear more distinctly.

In many intracerebral grafts, in the earlier as well as in the later stages, Bielschowsky's preparation showed also the presence of so called small nervous rings and of terminal swellings. These nervous formations, which have, especially in recent years, frequently arrested the attention of several authors, were situated in the cerebral parenchyma surrounding the grafts and very often at the periphery of the tumours themselves in the above-mentioned zones where also variously altered nervous cells and fibrils were found. The rings (Plate V Fig. 14) were generally very small, free, or, each at the end of a very fine fibril, scattered in the tissue without any order; they were more numerous in the earliest stages (Plate V Fig. 13 and 14). The terminal swellings were found less frequently and especially in larger sized tumours; some of them appeared as real simple swellings at the end of fibrils of different thickness and were stained uniformly black according to Bielschowsky's method; others were provided with a lighter little capsule in which very minute threads could be seen.

Something of the same kind was also noticed in the above-mentioned human „sarcoma pontis"; only rings were not found and the terminal swellings were relatively rare. In this case there were noticed a few interesting formations consisting of a little compact mass provided with a lighter capsule and out of which two thick fibrils went into opposite directions (Plate IV Fig. 7).

All these alterations may be explained as stages in a process of slow atrophy of the brain parenchyma, being a result of the progressive growth of the tumours, and they themselves prove that the nervous elements have a great resistance against the destructive power of the sarcomatous cells. Otherwise it would be impossible to explain the presence of nervous cells, comparatively little changed, in parts of the tumours themselves. As to the presence of small rings and terminal swellings in the immediate neighhourhood and at the periphery of the grafts, only the simple statement of the fact is made without detailed description, because it is a question, „mutatis mutandis" of well known things and because the debate about their significance did not lead to any satisfactory conclusion. It seems useful to insist on one point only: viz. the presence of small rings already in the earliest stages, i. e. three days after inoculation. These interesting and curious nervous formations

are, as is well known, considered by several athors as a sign of regeneration on the part of the nervous fibrils: from this arises the interesting problem whether here also we may speak of appearances of regeneration considering that these formations were observed very soon after the inoculation of a sarcoma and in the immediate proximity of well growing grafts.

Reverting to the intracerebral grafts of rat sarcoma, it remains to be considered whether their progressive growth, apart from the luxurious new formation of blood capillaries, also provokes a special reaction in the surrounding tissue, and of what nature this reaction would be. In the first days after transplantation there may be found immigration of a certain number of polymorphonuclear leucocytes [1]), the great majority of which degenerate; only very few seem to remain as permanent elements of the very thin and delicate stroma of the new tumour. A few polymorphonuclear leucocytes may be noticed also at the periphery of larger-sized tumours and some in the small areas of necrosis, being probably the result of a limited secondary immigration during the growth of the tumours, as in subcutaneous grafts; although in the intracerebral tumours the number of leucocytes really seems to be smaller.

Some lymphocytes were observed only in the earlier stages and very few afterwards in the larger sized tumours. So far the reaction differs enough remarkably from that seen in subcutaneous grafts in normal animals, as in the latter the lymphocytes appear in limited but perceptible number.

The same may be said of other elements of mesodermic origin which, though only in a small measure, take a part in the formation of the stroma of tumours growing in the subcutaneous tissue: viz. the wandering cells, the eosinophile leucocytes, the „Mastzellen" and the „Plasmazellen", the search for which was always negative. In other words, the propagation of a transplantable malignant new growth (sarcoma) into the brain of normal animals of the same species, provokes apparently a much less intense reaction than in the subcutaneous tissue.

In intracerebral grafts, on the contrary, a comparatively great number of so-called „Gitterzellen" occurs with some frequency. It is more rational however to connect these elements with the unavoidable haemorrhage caused by the needle penetrating into the nervous parenchyma than with the progressive growth of the tumours. This explanation is

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[1]) I have used, also in this paper, for the leucocytes, lymphocytes etc., the nomenclature adopted in my „Zelluläre Analyse der Geschwulstimmunitätsreaktionen". Zeitschr. f. Immunitätsf. Bd. V. H. I., 1910.

confirmed by the greater number found in the early stages, by their presence especially in those places where small quantities of blood are also found, and by the existence in their protoplasm of easily recognisable blood corpuscles. No detailed discussion will be attempted of whether the „Gitterzellen" are quite identical with the macrophages, which are found in subcutaneous tumours, e. g. after an injection of adrenalin solution, during spontaneous absorption etc. There are no noticeable morphological differences between „Gitterzellen" and macrophages. But there is still some disagreement as to whether the macrophages are elements of haematogenic or histogenic origin. The „Gitterzellen" have been derived respectively by different authors from the „Adventitialzellen" (Marchand), from the endothelium of the blood-vessels, from the blood itself, from the neuroglia.

Many difficulties were presented by the study of the alteration of the neuroglia in the brain parenchyma surrounding the grafts of rat sarcoma. The difficulties could be only partially overcome. It seems doubtless that in the first days after implantation the neuroglia showed phenomena of reaction of a progressive character. Here and there, a noticeable number of neuroglia cells were observed with clearly enlarged protoplasm, and with prominent light nuclei, which were also a little bigger than the nuclei of unaltered cells, at the periphery of the smaller grafts (i. e. after 3 days), in the proximity of the secondary nodules, and also in those places where there was only a proliferation of the endothelial cells and a new formation of blood capillaries. The form of the protoplasm, the arrangement of the prolongations, the aspect of the nucleus, and the evident relation to the blood-vessel supply taken all together suffice to characterize these elements and to remove every doubt about their nature. The uncertainty refers to what will become of these evidently hypertrophic neuroglia-cells and to the behaviour of the neuroglia in the later stages. Already in the five days old tumours which are still small, we are surprised to find hardly any more hypertrophic neuroglia-cells in the immediate neighbourhood of the sarcomatous elements, whilst the phenomenon persists in zones a little more peripheral and in the proximity of smaller secondary nodules. In those zones, which are in immediate contact with the growing tumour, where in the preceding period we noticed the hypertrophic neuroglia cells, we now remark quite the opposite phenomenon, i.e., the size of the same cells diminishes whereas their number seems to increase. One of the figures (Plate VI Fig. 15) illustrates this clearly; it was drawn from a 4-days old graft in a place where the limit between tumour and nervous tissue was quite distinct. In the places farther away from the tumour there are to be seen some enlarged neuroglia cells; in the neighbourhood of and between the sarcoma cells

themselves are other neuroglia cells, but smaller, with more uncertain outlines and thinner protoplasm; the nuclei have lost their original roundish form, and appear lengthened in different directions and indented in places so as almost to suggest incomplete phases of amitosis. In some of such cells vacuolisation also occurs. In other parts of this early tumour and also in tumours more developed, the same phenomenon is to be found, but characterised also by the presence of little groups of neuroglia cells, the size of which was still more reduced, the nuclei extremely pale and hardly recognisable (Plate VI Fig. 16). In those places of the tumour represented in Plate III Fig. 2, where it passes into the nervous tissue without exact demarcation, there are many of these small neuroglia cells mixed up with the sarcoma elements.

These observations are the result of the careful study of preparations stained almost only according to Nissl's method. To control them attempts were made to utilise other methods of neuroglia staining, but without reaching any definite result. All the methods proposed have given results from which it is impossible to draw positive conclusions. One observation has a negative value; the neuroglia fibres in the proximity of the elements of the tumours seemed to have lost their capacity for staining. Conclusions drawn from negative results on staining neuroglia are apt to be fallacious and not much weight can be attached to this observation.

Summarising the last few pages it seems likely that as a consequence of the intracerebral inoculation of a sarcoma (J. R. S.) the neuroglia at first shows some phenomena of reaction consisting in slight hypertrophy of the cells and limited hyperplasia. But with the progressive growth of the tumour the reaction instead of becoming more intense diminishes and the hypertrophic cells in the immediate neighbourhood of the graft slowly dwindle away. At the same time in more remote places, probably in consequence of the dying away of finer nervous structures, new neuroglia cells hypertrophy, but atrophy again because of the increase of the sarcoma. In this way the whole process would repeat itself and it would be clear enough why there did not exist any sign of sclerosis or of augmentation of neuroglia fibres at the periphery of very big tumours, i. e., many days after transplantation.

Can the above described facts be brought into relation with the rate of growth of the tumour used for intracerebral propagation? It was natural at first, to think that the absence of any lasting reaction on the part of the neuroglia might be the consequence of too rapid a growth of the tumours; in other words that no neuroglia proliferation had occurred around the neoplasms because there was not sufficient time for it. The comparison however with other tumours at once showed that this assumption would be wrong. No neuroglia proliferation could be

demonstrated around the grafts of mouse carcinoma „T" and rat carcinoma „F. R. C.". The former really grew a little more quickly, but the latter much more slowly than the rat sarcoma. If the slower growth were accompanied by neuroglia reaction, it should have been found, at all events in the latter case, but the preparations on the contrary showed a slighter reaction of the neuroglia than with the rat sarcoma. The presence or absence of neuroglia proliferation, depends therefore on other factors which are not yet fully demonstrated, but whose existence may at least be suspected.

They are suggested by the examination of the above-mentioned intracerebral grafts of the venereal tumour of the dog. It seems convenient at present to omit every other detail regarding the intracerebral growth of this tumour for the purpose of dwelling upon one only, which bears especially on the preceding discussion. The little tumour was developing in the third ventricle of a dog and its point of implantation corresponded with the site of the „nucleus medialis" of the left half of the optic thalamus and lay on the same height as the posterior commissure. The tumour penetrated some way into the tissue of the optic thalamus. The limit between thalamus and tumour was quite indistinct and there the following elements were intermingled: (1) roundish elements which provisionally may be looked upon partly as tumour cells, partly as lymphocytes and plasma cells; (2) easily recognisable neroglia-cells of different shapes and sizes, with prolongations in different directions. They now surrounded thickened blood-vessels with which they were sometimes connected by the prolongations, or, mingled with the above mentioned roundish elements. Two pictures (Plate VI Figs. 18 and 19) from two different places illustrate the above-mentioned facts. One (Plate VI Fig. 18) shows a blood-vessel surrounded by a extraordinarily enlarged neuroglia cell; both give a good idea of the complicated arrangement of the hypertrophied neuroglia cells intermingled with the tumour elements, lymphocytes and plasma cells. In the deeper parts, where the elements of the tumour gradually become fewer, there still appeared hypertrophied neuroglia cells of different shapes. Some tumour cells lay all over the surface of the optic thalamus in irregular groups and penetrated in between the fibres of the „stratum zonale". Around these tumour cells there was again an active proliferation of neuroglia cells to be seen. So there was no doubt here about a strong reaction on the part of the neuroglia.

It is true that in this case the host was quite different (dog) and the tumour grew very slowly. The site of implantation was also different and perhaps more suitable for the recognition of a neuroglia reaction; still the difference between this reaction in the dog and those observed in the mouse and rat is too considerable to be explained by these reasons only.

It is quite rational to suppose, that the most important factor in producing the phenomenon, is to be found in the different nature of the tumours used for intracerebral propagation. In fact the rat sarcoma ("J. R. S."), the mouse carcinoma („T",) and the rat carcinoma ("F. R. C.") are really malignant new growths in the strict meaning of the word, whereas the nature of the dog tumour, held by Sticker to be a lympho-sarcoma, cannot be looked upon as definitely established, and it is still considered to be a venereal granuloma by Bashford.

At present it suffices to demonstrate that when a tumour is transplanted into the brain, it may or may not call forth a glia reaction, according to its nature, and this fact is one of the values of these intracerebral inoculations. If it were possible to prove the above mentioned fact for a number of other tumours, we might turn the matter round and employ intracerebral inoculation to determine the nature of some tumours, as an addition to other experimental methods.

III. Intracerebral Propagation of Mouse Carcinoma ("T").

Of the two carcinomata used for intracerebral propagation, carcinoma „T" is that which presented the least interesting features. In a first series of experiments 8 young mice were employed; in only one a comparatively small tumour was obtained 15 days after the operation. The graft, taken out of the surrounding tissue as well as possible, was employed to inoculate a second series of 8 mice, which were killed, two at a time, 6, 9, 12 and 15 days after transplantation. The result was positive in all these animals.

In all the grafts, carcinoma „T" appeared as a solid carcinoma consisting of islands of large epithelial cells lying closely together, without lumina, and separated by an extraordinarily delicate stroma formed of quite fine thin-walled blood-vessels. Compared with the results of intracerebral propagation of the rat sarcoma, the most important facts noticed in grafts of carcinoma „T", were the expansive growth and the absence of any reaction on the part of the surrounding tissue (Plate III Fig. 1).

In the early, as well as in the later stages, the grafts always appeared as regularly shaped roundish tumours distinctly separated from the surrounding tissue; no formation of secondary nodules, no infiltration along the blood-vessel supply. There was hardly any sign of reaction in the nervous parenchyma. Special attention was paid to the way in which the delicate stroma of the tumours had originated; the phenomenon however remained rather obscure; in all the tumours it was possible to

distinguish here and there small capillaries penetrating from the surrounding tissue into the graft, but this was to be observed with certainty in few preparations, and the new formation of blood capillaries always remained small and was certainly not to be compared with what we have seen in the intracerebral graft of the rat sarcoma. The deep situation of the tumours in the brain (Plate III Fig. 1) excludes the supposition that the stroma might have its origin in the meninges. In spite of the scarcity of directly observed data the view appears justified that the delicate stroma of carcinoma „T" grafts originates entirely from the blood supply of the brain.

In the first days after transplantation some polymorphonuclear leucocytes were noticed in the neighbourhood of the grafts of carcinoma „T", but they soon disappeared. Some accumulations of small lymphocytes were also observed here and there especially in the younger tumours. Their presence has but limited value because in the second series of intracerebral propagation of carcinoma „T", particles of contused brain parenchyma were involuntarily inoculated together with the tumour cells. It is very probable that these particles, having become necrotic, provoked a limited and circumscribed reaction of lymphocytes. Of other elements, such as eosinophile leucocytes, plasma cells, etc., no trace could be found. Some „Gitterzellen" were present in the place where the needle, penetrating into the brain parenchyma, had provoked very minute micros-copical haemorrhages.

Very few observations were made as to the nervous elements, since Bielschowsky's method did not in this case give satisfactory results. On the other hand the expansive growth of the tumour provoked only the phenomena of compression referred to below in describing tumours developed extracerebrally.

IV. Intracerebral Propagation of Rat Carcinoma. (F. R. C.)

Two series of six animals each were employed. As the rats of the first series were killed too soon (between 2 and 15 days) they are left out of account. The microscopical examination however proved that the result had been positive, but the tumours were very small. In a second series, the rats were killed 6, 13 and 56 days after propagation, two at a time. The result was always positive, but the growth was extraordinarily slow.

The 6-day old graft could be recognised only with the help of the microscope. It was formed of little groups of epithelial cells disposed at the periphery of a mass of necrotic connective tissue which, with great probability, represents a dead residue of original tumour's stroma transplanted involuntarily with the carcinoma cells. The graft had penetrated into the Cornu Ammonis. No conclusions about the manner of growing could be drawn from this graft.

The 13-day old tumour was also very small but easy to recognise with the naked eye; its proportions were about the same as those of a graft of rat sarcoma 4 days after propagation. The small tumour was formed of a central mass of distinctly acinous structure and some secondary nodules scattered in the surrounding tussue, just as described for the early stages of rat sarcoma. The secondary nodules were composed of some epithelial cells lying closely together but, in this case, they did not seem to stand in direct relation to the blood vessel supply. In other words it was more a question of tumour prolongations penetrating into the surrounding tissue than of a really infiltrative growth along pre-existing blood vessels. An active new formation of blood capillaries however was also present and perhaps more luxuriant than in the neighbourhood of the sarcoma grafts. The brain parenchyma close to the tumour was here crossed in different directions by rather dilated, and evidently newly formed blood capillaries which were constituted by a simple layer of endothelial cells. In other places some newly formed capillaries were penetrating into the principal tumour mass but much more clearly in this case than in the graft of carcinoma „T". This fact and the deep situation of the little tumour in the brain lead to the conclusion that the stroma of the new tumour originates from the cerebral blood vessels.

The grafts of rat carcinoma were always much richer in supporting tissue than those of mouse carcinoma „T". This fact however, is easy to understand when considering that the rat carcinoma had a very abundant stroma at the time of transplanting it intracerebrally. It is doubtles that the stroma transplanted with the graft degenerates; but we may admit that the nature of the transplanted cells have an influence in determinating the amount and arrangement of the stroma, supplied afresh from the host tissue.

In the above described grafts of rat carcinoma and in the others too, there was noticed a much greater number of polymorphonuclear leucocytes than in other intracerebral tumours. This must be referred to the large extent of the necrosis, which in this case was very abundant in the smaller as well as in the larger sized tumours.

Together with the polymorphonuclear leucocytes a limited number of lymphocytes was observed. A special reaction of other elements (plasma cells, "Mastzellen", etc.) was never noticed. There was a very variable quantity of „Gitterzellen" in the neighbourhood of the 13- and 6-day old graft, as well as in the following stage, associated as before with haemorrhages, so that the same reasons as adduced in Chapter II. with respect to the grafts of rat sarcoma explaine their presence.

The 13-day old graft was well fitted for the study of the alterations

of the nervous parenchyma according to Bielschowsky's method. The results were not really different from those obtained with rat sarcoma. The nerve cells also showed slowly advancing atrophy connected with great resistance on the part of the neurofibrils. In the brain parenchyma surrounding the carcinoma grafts and especially in the proximity of the secondary nodules there were also small rings (Fig. 20), but the terminal swellings found in the rat sarcoma were not detected. There were present moreover a noticeable number of so-called „plaquetas" (Cayal). For all these formations the considerations given above are valid.

The 56-day old tumour was a very marked example of a tumour of slow growth, and very interesting also because of its large size and way of spreading into the nervous tissue. As to the way of spreading it may be said, that it is a repetition only on a larger scale, of the facts described with respect to the 13-day old grafts. The central mass and the secondary nodules have now a much larger size and the destruction of brain parenchyma is naturally greater too. Carcinoma offshoots have penetrated in different directions into the nervous parenchyma. Here the epithelial cells are arranged in an absolutely different way. In some places they form a nearly regular layer of columnar epithelium, bent in several places, and enclosing a sort of cavity wherein necrosed cells and polymorphonuclear leucocytes are to be found. In Plate VI Fig. 17 is drawn part of this wall of lining epithelium: it illustrates the external disposition of carcinoma elements — in direct contact with the nervous tissue — supported by a very delicate basal membrane. In other places preponderates a characteristic arrangement in acini, the walls of which are typically formed by a single layer of epithelium, or atypically by several layers of superimposed cells. Together with the acinous arrangement the alveolar type of growth also occurs, where no lumina are present in the groups of epithelial cells.

The stroma was quite differently developed; in many places it seemed to be entirely wanting, in others it consisted only of very delicate strands of connective tissue carrying capillaries between the acini and the alveoli; in other places again there were well defined layers of connective tissue showing a great number of blood capillaries and a variable number of leucocytes and lymphocytes.

Corresponding to the irregularly developed stroma, i. e., to the irregularly developed blood supply, the necrosis had quite a different extension, so that necrotic areas and healthy layers of epithelial cells alternated irregularly. Moreover, in this tumour severe haemorrhage had occured, and consequently large areas of the tumour itself were infiltrated with red corpuscles, between which healthy epithelial cells were found arranged in groups and acini.

Careful investigations were made for the purpose of ascertaining if there was a reaction on the part of the neuroglia. The result was a negative one, as already described, though the conditions for it seemed particularly favourable. Plate VI Fig. 17 may serve as an illustration of the absence of every form of reaction: the carcinoma cells growing into the nervous parenchyma are in direct contact with it, but no hypertrophy or hyperplasia of the neuroglia elements can be remarked.

Pieces of the 56-day old rat carcinomata were treated according to Bielschowsky's block method in order to investigate the alteration of the nerve cells and nerve fibrils; but unfortunately the preparations were all unsuccessful, so that nothing certain can be said about this question.

V. Intracerebral Propagation of Rat Sarcoma („J. R. S.") in Immune Animals.

The facts noted in these preliminary observations are interesting in themselves, and also they demonstrate the importance of intracerebral propagation of malignant new growths for the accurate analysis of questions regarding the general problem of immunity against cancer. They have the advantage over the study of subcutaneous grafts in so far, that being remored without any disturbance of surrounding tissues the anatomical relations are better preserved.

Six rats in which a first subcutaneous inoculation of sarcoma had been followed by transitory growth and subsequent absorption 3—4 months before, were used for intracerebral inoculation with the intention of finding out whether a new graft could grow in the brain. The rats were about 6 months older than the normal control animals used for intracerebral propagation of sarcoma. The rats were killed 2, 3, 4, 6, 8 and 10 days after transplantation, i. e., one at a time. Only the 3 and 8-day old stages could be made object of careful examination. These two grafts with a wide margin of the surrounding tissue were cut in complete series.

The 3-day old stage (Fig. 21) had, with regard to size and spread, grown almost exactly like a graft of the same age in normal animals. But in the most central parts of the very small tumour there existed a rather large zone of necrosis where only few greatly altered nuclei were to be seen. The necrosed area had not the general aspect usually seen in large sized tumours as a consequence of insufficient blood supply, and there did not exist an abundant cellular detritus; the numerous polymorphonuclear leucocytes as observed e.g. in the necrotic areas of the intracerebral graft of rat carcinoma were also absent. The necrosed

area appeared in this case as a uniform, compact mass without any structure, due to a kind of coagulation necrosis. The sarcoma cells lay at the periphery of the zone of necrosis and were scattered in the surrounding nervous tissue in the shape of many secondary nodules. Many of them appeared in different phases of mitosis.

The investigation of the same preparation under great magnification revealed two other interesting facts: (1) The much less extensive formation of new blood capillaries in the surrounding nervous tissue, compared with what was seen in grafts of sarcoma in normal rats; (2) The presence of scanty but clearly recognisable infiltration of lymphocytes in the immediate neighbourhood of some newly formed capillaries. Very often the lymphocytes lay along the outside of the endothelial walls of the capillaries, which in some places were filled with lymphocytes too, just as happens during spontaneous absorption of subcutaneous grafts. These features were to be found especially in the central part of the small graft at the limit between necrotic and healthy tumour areas. In this case there were present undoubted signs of active growth (increase in bulk, mitosis, etc.) but at the same time from certain phenomena (central necrosis, diminished new formations of blood capillaries, infiltration of lymphocytes) it might be concluded that the conditions of growth differed from the normal and that the continued growth of the tumour might later on be hindered in some way or other.

The following stages proved that these suppositions were right. In the 8-day old graft the first thing attracting attention was the small size of the tumour. It was about the same size as the 3-day old tumour and much smaller than a graft of about the same age in a normal rat. Moreover few secondary nodules could be found in the surrounding tissue, and only in the immediate neighbourhood of the tumour. The central area of necrosis was again of unusually large extent and occupied nearly the whole tumour; only at the periphery there existed a thin layer of healthy sarcoma cells the greatest number of which were small and irregularly shaped; very few elements had preserved their original form, which however seemed to be very often enlarged. A few sarcoma cells still continued to divide mitotically. The whole graft had shrunk somewhat and a small cleft was produced between the tumour and the nervous tissue; a certain degree of shrinkage occurred also in the nodules scattered in the surrounding tissue.

Altogether the graft showed an extraordinary resemblance to the appearances described and illustrated by Russell in his paper „The Nature of Resistance to the Inoculation of Cancer". Russell inoculated carcinoma „27" and several other tumours into immune mice. But, apart from the different nature of the tumours used by me, a comparison is

quite instructive, since the histological pictures 4 and 6 of Russell's paper and my Fig. 22 Plate VII illustrate nearly identical phenomena. A large necrotic area occupies the whole of the graft; the healthy tumour cells lie at its periphery, where they are applied to the host tissue. Only in my case the extent of the healthy tumour zone is larger, and there had been a new formation of blood capillaries, which did not occur in Russell's grafts. But that is easy to understand. Russell used for his experiments highly immune mice, whilst I took for intracerebral propagation old rats, which had only once been inoculated unsuccessfully with sarcoma, i.e. animals which were but partially resistant in consequence of a preceding absorption of only a small amount of tumour tissue. The degree of immunity may have been strengthened by their age. Corresponding to this partial resistance, there had also been a certain growth and a limited new formation of blood capillaries already from the first days after transplantation. The new, little tumour therefore developed under about the same conditions as the first inoculation of a sporadic tumour in a normal animal, in which the graft grows for a short time and is afterwards absorbed.

These conclusions were proved by: (1) the presence of a noticeable number of healthy elements 3 days after propagation; (2) by the abovementioned infiltration of lymphocytes 3 days after propagation; (3) by the presence, in a few places of the 8-day old graft, of a distinct infiltration of plasma cells (Fig. 23 Plate VII). Amoug all the intracerebral propagations made with different tumours into rats and mice, this is the only circumstance in which plasma cells were observed. This fact cannot be without some significance; on the contrary it corresponds with the partial degree of resistance and confirms it. It also agrees with the conclusions arrived in a preceding paper on the study of the cellular reaction in the host tissues during the production of resistance against mouse carcinomata. The production of resistance was always preceded and accompanied by a reaction of lymphocytes and plasma cells. By grafting sarcoma into the brain of partially immune rats, the same phenomenon has been elicited; it must therefore have the same significance, namely, the production of a greater degree of immunity.

Finally, attention may be drawn to another fact, viz. the presence of a distinct proliferation of the neuroglia in places about the same as those where the reaction of plasma cells occurs (Fig. 23 Plate VII). The neuroglia proliferation is very significant in this combination. During the absorption of subcutaneous grafts the elements of the surrounding tissue proliferate and, at last, form a cicatrix, so also the supporting tissue of the brain proliferates to repeat probably the corresponding process in a different tissue.

It would be useless at present too insist to much on these different questions. It was not possible to follow the process in its entirety, and nothing certain can be said about what would have happened if the healthy elements of the tumour had conquered the reaction, or if they had been entirely absorbed. It was also impossible to undertake any special method for neuroglia staining. New researches as to the validity of these conclusions are more necessary than for those deduced from the other experiments mentioned. Still the facts have a certain value: — (1) They permit us to affirm that the resistance, following absorption of tumour tissue, even if partial, is a general phenomenon and becomes evident also in organs and tissues which differ entirely from the tissue in which the absorption of tumour cells has first taken place. (2) They show that the nervous tissue may be very valuable in the histological investigation of difficult and delicate points in the problem of resistance against cancer. This is proved by the clearness of the histological pictures, the ease with which the sarcoma cells could be distinguished from the products of reaction and from the nervous elements. The analysis of the process of sarcoma absorption in the subcutaneous tissue on the contrary, might present much greater difficulties. (3) Probably they offer an excellent method to provoke and investigate neuroglia proliferation under circumstances in which it proceeds slowly. It would be very interesting from this point of view to study the consequences of the absorption of tumours, which have attained a considerable size in the brain.

Summary.

A paper which bears the character of a series of still incomplete experiments, made according to an entirely new method, and about a difficult subject does not allow of final and general conclusions being drawn. Far more and extensive experiments must first be made. What follows is to be considered as a short summary of some facts which may be a stimulus for other investigators to do further work in the same direction.

With the above reservations I may state the following propositions: —

1. With a simple but appropriate technique it is possible to propagate transplantable new growths of rats, of mice and also of dogs in the brain of other animals of the same species. Intracerebral propagation succeeds with about the same percentage as subcutaneous transplantation. The energy of growth of intracerebral grafts seems however to be somewhat lower.

2. The growth of a tumour in the brain of a rat or a mouse does not provoke the manifestation of specific symptoms referable to a toxic effect of the cancer tissue; such symptoms as appear are merely distur-

bances of respiration and those appeared only in cases where the tumours had reached very enormous proportions compared with the small brains. Even the destruction of more than half of the brain was not followed by a real paralysis of the limbs of the opposite side of the body.

3. The type of growth of an intracerebral graft may be infiltrative or expansive or a combination of both; the mode of growth seems independent of the nature and histology of the transplanted tumours. The infiltrative type of growth of a sarcoma may disappear when the graft remains extracerebral. The tumours develop through multiplication of their parenchymal elements; the stroma originates from the blood supply of the brain.

4. The type of growth of a sarcoma of a rat when transplanted into the brain does not differ essentially from that of a spontaneous sarcoma of the human brain.

5. The spread of a sarcoma in the brain follows the blood supply of the surrounding nervous tissue. Carcinomata, if growing infiltratively, spread into the surrounding tissue, independent of the blood supply.

6. The cells of a spindle-cell sarcoma transplanted into the brain of a rat show transitorily a polymorphous condition, due probably to the somewhat altered conditions of existence.

7. Intracerebral propagation of a sarcoma provokes an abundant new formation of capillaries. This is generally less evident after transplantation of carcinomata.

8. Propagation of sarcomata and carinomata into the brain of normal rats and mice, does not provoke any special reaction on the part of elements of mesodermic nature. A reaction of plasma cells and lymphocytes appears only in partially immune animals.

9. As a consequence of the intracerebral propagation of tumours the nervous elements undergo a process of slow atrophy. The nervous fibrils show a very great resistance to the progressive encroachment of the tumour. As in other pathological processes of nervous tissue the fibrils do not disappear till the nerve cells have reached an extreme degree of atrophy.

10. A lasting proliferation of neuroglia seems to appear only under special conditions, which may be determined either by the nature of the propagated tumour or by the pre-existence of a partial degree of immunity in the host tissue.

11. Resistance against cancer which follows the absorption of tumour tissue is a general phenomenon, and manifested also in organs and tissues, which differ completely from the tissue in which the primary (immunising) absorption took place.

Description of figures.

PLATE III.

Fig. 1. Mouse Carcinoma („T"). intracerebral. The whole of a graft preserved 10 days after inoculation into the brain of a mouse. The tumour has grown into the optic Thalamus and has destroyed a small portion of the Cornu Ammonis. The new growth is sharply demarcated off from the surrounding nervous tissue. H. = Habenula. Cf. = Commissura posterior. CA. = Cornu Ammonis. Fd. = Fascia Dentata. Form. 10% Haem. Eos. Obj. 2, Oc. 1 Leitz.

Fig. 2. Rat sarcoma. („J. R. S.") Frontal section of the brain of a rat with a 17 day old graft. The figure shows the growing of the tumour in the cerebral cortex and somewhat extracerebrally. Partial destruction of the „Cornu Ammonis" of the left side. Compression of the Thalamus opticus. The tumour passes gradually into the nervous tissue. S. C. A. = Subiculum cornus Ammonis. N. l. = Nucleus lenticularis. C. int. = Caps. interna. C. ext. et extr. = Capsula externa et extrema. C. f. = Commissura fornicis. t. opt. = tractus opticus. N. p. D. F. ($X\frac{1,4}{1}$).

Fig. 3. Rat sarcoma, („J. R. S.") 27 A. Sagittal section of the left hemisphere of the brain of a rat to show the great extension of the tumour. A. = Alveus. H. = Hippocampus. F. h. = Fissura hippocampi. F. d. = Fascia dentata. F. = fimbria. Form 10%. Weigert's Iron Haematoxylin. v. Gieson. (Edinger Apparatus.)

Fig. 4. Rat carcinoma („F. R. C.") 32 A. Frontal section of a rat's brain showing a graft of Flexner's carcinoma 56 days after inoculation. Growth in part extracerebral. A small part of the tumour was cut off with the skull. Destruction of a great part of the cortex, the Cornu Ammonis, the optic thalamus of the right side, and of a great part of the optic thalamus of the opposite hemisphere.

C. c. = Corpus callosum. C. f. = Commissura fornicis. S. C. A. = Subiculum Cornus Ammonis. F. d. = Fascia dentata F. = Fimbria N. c. = Nucleus Caudatus. Th. opt. = Thalamus opticus. C. ant. = Commissura anterior. V. l. = Ventriculus lateralis. Form 10%. Haem. Eos. Obj. a^2 Oc. 2 ($X\frac{1,5}{1}$).

PLATE IV.

Fig. 5. Isher, intracerebral. The whole of a graft of a Sticker's venereal dog tumour preserved one month after inoculation into the brain of a dog. The tumour was implanted into the left side of the optic thalamus and has extended into the III ventricle.

C. g. lat. dors. = Corpus geniculatum laterale-dorsale. C. g. lat. ventr. = corpus geniculatum laterale-ventrale. N. v. = Nucleus ventralis. C. A. = Cornu Ammonis. F. h. = Fissura hippocampi. — F. = Fimbria. C. s. h. = Commissura supra-habenularis. C. post. = Commissura posterior.

Form. 10%. Haem. Eos. (Edinger Apparatus.)

Fig. 6. Rat sarcoma. („J. R. S.") 29 B. The whole of a graft preserved 4 days after implantation. To illustrate the infiltrative growth of rat sarcoma into the brain. (a) central mass. (b) secondary nodules arranged along blood capillaries. (c) area of necrosis. Alc. Azur. II. Obj. 2. Oc. 1 L.

Fig. 7. Sarcoma of Pons. (Human). Infiltrative growth of a human sarcoma.

(a) = Healthy nerve cell; (b) = atrophied nerve cell; (c) = sarcoma elements; (d) = swelling with two prolongations and a cowl: (strongly altered nerve cell?); (e) = neuroglia cell; Form. 20%. Bielschowsky's method. Obj. 3 m.m. Oc. 6. comp. Z. ($X\frac{500}{1}$).

Fig. 8. Rat sarcoma. („J. R. S") 29 B. intracerebral. From a graft of sarcoma preserved 4 days after implantation into the brain of a rat. Polymorphcelled stage characterized by enlarged sarcoma-cells. Borrel. Iron Haem. Obj. 3 m.m. Oc. 6. comp. Z. ($X\frac{500}{1}$).

Fig. 9. Rat sarcoma. („J. R. S.") 29 B. intracerebral. From a graft preserved 4 days after implantation into the brain of a rat. Polymorphcelled appearance. Group of irregularly shaped *small* sarcoma cells. Alc. Azur. II. Obj. $^1/_{15}$ Oc. 4. comp. Kor. ($X\frac{600}{1}$).

PLATE V.

Fig. 10. Rat. sarcoma. („J. R. S.") intracerebral. Large sarcoma cell (a) in the nervous parenchyma at a comparatively great distance from the implantation point of the graft. (b) = another sarcoma cell; (c) = neuroglia elements; (d) = red blood corpuscles; (e) = somewhat altered nerve tissue (beginning to degenerate); (f) = healthy nervous tissue; (g) = blood capillary. Borrel. Iron. Haem. Obj. 3 m.m. Oc. 6 comp. Z. ($X\frac{500}{1}$).

Fig. 11. Rat sarcoma. („J. R. S.") intracerebral. From a graft preserved 4 days after implantation into the brain of a rat. To illustrate the growth of the sarcoma cells along the capillaries.

E. = Endothelium. S. = Sarcoma cells. N. = Nerve cell. n. = neuroglia cell. Alc. Azur. II. Obj. D.D. Oc. 3 Z. ($X\frac{320}{1}$).

Fig. 12. Rat sarcoma. („J. R. S.") 27 B. intracerebral. From a graft of rat sarcoma preserved 4 days after implantation into the brain of a rat. Secondary nodule. The fig. shows the new formation of blood capillaries (a); the hypertrophy of some neuroglia cells, (b); sarcoma cells dividing mitotically, (c); different shaped sarcoma cells, (d). Alc. Azur. II Obj. 3 m.m. Oc. 6 comp. Z. ($X\frac{500}{1}$).

Fig. 13). Rat sarcoma. („J. R. S.") intracerebral. 17 days after propagation. (a) = Typically shaped sarcoma cells growing along blood capillaries (a^1); (b) = hypertrophied neuroglia cells; (c) = lymphocytes; (d) = atrophied nerve cell; (e) = terminal swellings; (f) = sarcoma cells arranged in a group. Form. 10% Bielschowsky's method. Obj. 2 m.m. Oc. 6 comp. Z. ($X\frac{250}{1}$).

Fig. 14. Rat sarcoma. („J. R. S.") intracerebral. 17 days after implantation. (s) = sacroma cells. (r) = small rings. (t) = big terminal swelling. (g) = neuroglia cells. Form. 10% Bielschowsky's method. Obj. $^1/_{15}$ Oc. 6 comp. Kor. ($X\frac{900}{1}$).

PLATE VI.

Fig. 15. Rat sarcoma. („J. R. S.") 29 B. intracerebral. From the periphery of a 4 days old graft to show the arrangement of the neuroglia cells in the neighbourhood of the sarcoma cells. (a) = hypertrophied neuroglia cells; (b) = smaller neuroglia cells with irregularly shaped nuclei; (c) = neuroglia cell with vacuole; (d) = sarcoma cells; (e) = „Gitterzellen"; (f) = polymorphonuclear leucocytes. Alc. Azur II. Obj. 3 m.m. Oc. 6. comp. Z. ($X\frac{500}{1}$).

Fig. 16. Rat sarcoma. („J. R. S.") 29 B. intracerebral. From a 4 days old graft, to show stronger atrophy and degeneration of neuroglia cells. (s) = sarcoma cells. (g) = neuroglia cells. Alc. Azur. II. Obj. $^1/_{15}$ Oc. 6 comp. Kor. ($X\frac{900}{1}$).

Fig. 17. Rat carcinoma. („F.R.C.") 32 A. intracerebral. From a 56 day old graft. Part of the wall of a cavity surrounded by a healthy epithelium growing into the nervous parenchyma, which appears quite unaltered. Inside polymorphonuclear leucocytes. Form. 10 °/₀ Haem. Eos. Obj. 3 m.m. Oc. 6 comp. Z. ($X\frac{500}{1}$).

Fig. 18. Isher, intracerebral. From a graft of a venereal dog tumour preserved one month after inoculation into the brain of a dog. To show the strong reaction of the neuroglia at the implantation point. (g) = hypertrophied neuroglia cells. (t) = tumour cells. (V) = thickened blood vessel. (x) = cells of undefined nature. Form. 10°/₀ Hoem. Eos. Obj. $^1/_{15}$ Oc. 6 comp. Kor. ($X\frac{900}{1}$).

Fig. 19. From the same preparation as Fig. 18. To show the strong neuroglia reaction and the presence of plasma cells and lymphocytes at the implantation point. (l) = lymphocytes. (t) = tumour cells. (P) = plasma cells. (g) = hypertrophied neuroglia cells. (x) = elements of unknown origin. ($X\frac{900}{1}$).

Fig. 20. Rat carcinoma („F.R.C.") 32 A. From the periphery of a graft of rat carcinoma preserved 13 days after propagation into the brain of a rat. (a) = carcinoma cells; (b) = neuroglia cells; (c) = altered nervous cells; (d) = polymorphonuclear leucocytes; (e) = small ring; (f) = plaquetas (?) Form. 10 °/₀. Bielschowsky's method. Obj. 2 m.m. Oc. 6. comp. Z. ($X\frac{750}{1}$).

PLATE VII.

Fig. 21. Rat sarcoma. („J.R.S.") intracerebral. From a graft preserved 3 days after inoculation into the brain of an immune rat. To show the rapid formation of a central necrotic area. (a) = healthy sarcoma cells. (b) = necrotic area. Form. 10 °/₀. Iron Haem. ($X\frac{16}{1}$).

Fig. 22. Rat sarcoma. („J.R.S.") intracerebral. From a graft preserved 8 days after inoculation into the brain of an immune rat. The tumour has not grown; the central part is necrotic. At the periphery of the tumour an evident reaction. Form. 10 °/₀. Iron Haem. ($X\frac{16}{1}$).

Fig. 23. From the same preparation as Fig. 22. Under greater magnification to show the reaction at the periphery of the necrotic area.

(N) = Necrotic area. (S) = healthy sarcoma cells. (P) = plasma cells. (g) = neuroglia cells. (l) = lymphocytes.

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